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(54) Title: THEREAPEUTIC APPLICATIONS FOR THE ANTI-T-BAM (CD40-L) MONOCLONAL ANTIBODY 5c8

(57) Abstract

Activation of cells bearing CD40 on their cell surface by CD40 ligand is inhibited by contacting the cells with an agent capable of inhibiting interaction between CD40 ligand and the cells, in an amount effective to inhibit activation of the cells. Activation of cells bearing CD40 on their surface by CD40 ligand in a subject is inhibited by administering to the subject an agent capable of inhibiting interaction between CD40 ligand and the cells, in an amount effective to inhibit activation of the cells. Conditions dependent on CD40 ligand-induced activation of CD40-bearing cells are treated.

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THERAPEUTIC APPLICATIONS FOR THE ANTI-T-BAM (CD40-L) MONOCLONAL ANTIBODY 5c8

This application claims the priority of U.S. Serial No. 08/567,391, filed December 1, 1995, and U.S. Serial No. 08/566,258, filed December 1, 1995 and U.S. Serial No. 08/637,323, filed April 22, 1996 the contents of which are hereby incorporated by reference into the present application.

The invention disclosed herein was made with Government support under NIH Grant Nos. K08-AR-01904, RO1-CA55713, RO1-AI-28367, RO1-AI-14969, HL21006, HL42833, HL50629, and RO1-AI-14969 from the Department of Health and Human Services. Accordingly, the U.S. Government has certain rights in this invention.

Throughout this application, various references are referred to within parenthesis. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found in the text or at the end of this application, preceding the sequence listing and claims.

Background of the Invention

CD40 is a 50 kDa cell surface molecule originally described as being expressed on B cells and some epithelial carcinomas (1, 2). CD40 interacts with CD40L (T-BAM, gp39, TRAP), a 30 kDa cell surface molecule transiently expressed on activated $CD4^{+}$ T cells (3-8). CD40L-CD40 interactions have been extensively studied in the context of T cell-B cell interactions. CD40 ligation plays key roles in B cell activation, proliferation, differentiation, Ig production and rescue from apoptotic signals (9-11). The critical in vivo role of CD40 ligation in B cell differentiation is highlighted by the hyper-IgM syndrome, a humoral immunodeficiency due to

mutations in the gene encoding CD40L (12-16). Murine CD40 (17) or CD40L (18) "knockouts" have similar phenotypes to patients with the hyper-IgM syndrome.

Interestingly, recent studies indicate that CD40 expression has a broader cellular distribution than originally described. CD40 has been shown to be expressed on monocytes (19), dendritic cells (22), epithelium (23, 21), basophils (24), and Hodgkin's tumor cells (25). Moreover, various cytokines can regulate CD40 expression on non-B cells. CD40 expression on thymic epithelial cells is upregulated by IL-1α, TNF-α or INF-γ (21). INF-γ, in addition to IL-3 or GM-CSF, similarly upregulates CD40 expression on monocytes (19). Ligation of CD40 in the presence of INF-γ and IL-1α stimulates GM-CSF production by thymic epithelial cells (21). In addition, CD40L expressing transfectants induce tumoricidal activity by monocytes and, in the presence of INF-γ, GM-CSF or IL-3, stimulate monocytes to secrete TNF-α, IL-6 or IL-8 (19).

CD40 is also expressed on cells found within synovial membrane (SM) in patients afflicted with rheumatoid arthritis (RA). An immunohistological survey of cell surface molecules expressed in RA SM found that CD40 was expressed on a variety of cell types, including cells with fibroblast-like morphology (26). In this report it is shown by FACS analysis that CD40 is expressed on cultured synovial membrane (SM) fibroblasts isolated from patients with RA, non-RA inflammatory arthritis (IA) or osteoarthritis (OA). In addition, dermal fibroblasts isolated from normal donors also express CD40. Moreover, CD40 ligation by CD40L^{+} cells induces fibroblast activation and proliferation.

Endothelial cells express surface molecules, such as CD54 (ICAM-1), CD62E (E-selectin) and CD106 (VCAM-1), that

mediate adhesive interactions with leukocytes (27-35). The expression of endothelial cell surface adhesion molecules orchestrates recruitment of leukocytes to sites of inflammation and therefore is subject to tight regulation (27, 28). Resting endothelial cells express low levels of CD54 and minimal or no CD62E or CD106. Following activation with IL-1, TNFα, or LPS, endothelial cells rapidly upregulate CD54, CD62E and CD106 expression (27, 28). $CD4^+$ T cells may contribute to upregulation of endothelial cell surface adhesion molecules by inducing endothelial cells or other target cells to secrete IL-1 or TNFα (36). However, the molecular details involved in $CD4^+$ T cell-endothelial cell interactions that induce endothelial cell activation have not been completely delineated.

It can now be reported that normal human endothelial cells also express CD40 <u>in situ</u> and CD40L-CD40 interactions induce endothelial cell activation <u>in vitro</u>. Frozen sections from normal spleen, thyroid, skin, muscle, kidney, lung or umbilical cord were studied for CD40 expression by immunohistochemistry. Endothelial cells from all tissues studied express CD40 <u>in situ</u>. Moreover, human umbilical vein endothelial cells (HUVEC) express CD40 <u>in vitro</u> and rIFN-γ induces HUVEC CD40 upregulation. CD40 expression on HUVEC is functionally significant because $CD40L^+$ Jurkat T cells upregulate HUVEC CD54 (ICAM-1), CD62E (E-selectin) and CD106 (VCAM-1) expression <u>in vitro</u> in a manner inhibited by anti-CD40L mAb 5C8. Additionally, CD40L expressing 293 kidney cell transfectants, but not control transfectants, also upregulate CD54, CD62E and CD106 expression on HUVEC. These results demonstrate that CD40L-CD40 interactions induce endothelial cell activation <u>in vitro</u>. It is shown for the first time that CD40L expressed on the surface of T cells induces activation of CD40+ endothelial cells and that this activation is inhibited by an anti-CD40L

monoclonal antibody. Moreover, these results demonstrate a mechanism by which activated $CD4^{+}$ T cells augment inflammatory responses _in vivo_ by upregulating the expression of endothelial cell surface adhesion molecules.

Summary of the Invention

This invention provides a method of inhibiting activation by CD40 ligand of cells bearing CD40 on the cell surface, comprising contacting the cells with an agent capable of inhibiting interaction between CD40 ligand and the cells, in an amount effective to inhibit activation of the cells.

This invention provides a method of inhibiting activation by CD40 ligand of cells bearing CD40 on the cell surface, in a subject, comprising administering to the subject an agent capable of inhibiting interaction between CD40 ligand and the cells, in an amount effective to inhibit activation of the cells in the subject.

Description of the Figures

Figure 1. CD40 expression on SM fibroblasts. Shown are FACS analyses of CD40, CD14, CD45 or MHC Class II expression, as indicated, on representative RA or OA SM adherent cells following the first passage in vitro. The X-axis represents mean fluorescence intensity (MFI) and the Y-axis represents cell number. For RA cells, the MFI of CD40 expression or isotype control mAb was 21 and 9, respectively. For OA cells, the MFI of CD40 expression or isotype control mAb was 33 and 9, respectively.

Figure 2. CD40 expression on resting or rINF-γ stimulated dermal fibroblasts. Shown are FACS analyses of CD40, CD54 or control mAb staining, as indicated, on 3 dermal fibroblast lines. The cells were cultured in the presence or absence of rINF-γ (1000 U/ml) for 24 hours. SK.1 and SK.2 were studied following the second passage and CCD 965 SK was studied following the third passage in culture. The X-axis represents mean fluorescence intensity (MFI) and the Y-axis represents cell number. The number in the upper right hand corner of each graph indicates CD40 MFI (background subtracted).

Figure 3. Cytokine regulation of SM fibroblast CD40 expression. Shown is a bar graph representing CD40 mean fluorescence intensity (MFI) on a SM fibroblast line (OA.3) following co-culture with rINF-γ (1000 U/ml), rIL-1α (10 pg/ml), rTNF-α (200 U/ml) or combinations of cytokines, as indicated. CD40 expression was determined by FACS analysis and background staining with a control mAb is subtracted for each value. The experiment shown is representative of 3 similar experiments performed.

Figure 4. Effect of CD40L-CD40 interactions on SM fibroblast CD54 (ICAM-1) expression. Shown are two-color

contour graphs demonstrating CD13 expression (X-axis) or CD54 expression (Y-axis) on IA.1 SM fibroblasts cultured 24 hours with media, rINF-γ (1000 U/ml), CD40L$^-$ Jurkat B2.7 cells or CD40L$^+$ Jurkat D1.1 cells in the presence or absence of anti-CD40L mAb 5C8 or control mAb P1.17. The number in the upper right hand corner of each graph represents CD54 mean fluorescence intensity (MFI). The background MFI of an isotype control mAb is subtracted from each value. The experiment shown is representative of 3 similar experiments performed.

Figure 5. Transfection of CD40L confers the capacity to upregulate SM fibroblast CD54 (ICAM-1) and CD106 (VCAM-1) expression. Shown are bar graphs indicating CD54 or CD106 MFI on SM fibroblasts following culture for 24 hours with media, CD40L$^+$ D1.1 cells, CD40L$^-$ B2.7 cells or CD40L$^+$ B2.7 transfectants, as indicated. CD54 and CD106 expression were determined by two-color FACS analysis as in figure 4. The background MFI of an isotype control mAb is subtracted from each value. The experiment shown is representative of 2 similar experiments performed.

Figure 6A. Effect of CD40L-CD40 interactions on fibroblast IL-6 secretion. Shown are bar graphs indicating ^{3}H-thymidine incorporation by the IL-6 indicator cell line B9 following the additions of supernatants (final dilution 1:60) from SM fibroblasts cultured with media alone, CD40L$^+$ D1.1 cells in the presence or absence of anti-CD40L mAb 5C8 or control mAb P1.17, CD40L$^-$ B2.7 cells or CD40L$^+$ B2.7 transfectants. The proliferative responses of B9 cells cultured with control supernatants from D1.1 cells, B2.7 cells or CD40L$^+$ B2.7 transfectants were 1136 cpm (± 113), 2398 cpm (± 263) and 1131 cpm (± 56). Similar results were obtained with 3 additional SM fibroblast lines.

Figure 6B. B9 proliferation in response to rIL-6. In a parallel experiment to that shown in figure 6A, B9 cells were cultured with varying concentrations of rIL-6.

Figure 7. Effect of CD40 ligation on SM fibroblast proliferation. Shown are bar graphs from 2 separate experiments demonstrating SM fibroblast ^{3}H-thymidine incorporation following coculture in 1% FM with mitomycin-C treated CD40L$^-$ Jurkat B2.7 cells or CD40L$^+$ Jurkat B2.7 transfectants for 48 hours. Where indicated, CD40L$^+$ Jurkat B2.7 transfectants were pretreated with anti-CD40L mAb 5C8 (5 μg/ml) or P1.17 control mAb (5 μg/ml) prior to the addition to fibroblasts. In the experiment studying RA.5 proliferation, the proliferation of CD40L$^-$ Jurkat B2.7 cells or CD40L$^+$ Jurkat B2.7 transfectants was 51 ± 7 cpm and 39 ± 3 cpm, respectively. In the experiment studying OA.6 proliferation, the proliferation of CD40L$^-$ Jurkat B2.7 cells or CD40L$^+$ Jurkat B2.7 transfectants was 243 ± 5 cpm and 453 ± 95 cpm, respectively. Background proliferation is subtracted in coculture experiments. Also shown are the proliferative responses of fibroblasts following culture in 1% FM or 10% FM. Similar results were obtained in 3 additional experiments. Error bars show observed error.

Figure 8. Effect of rINF-γ on CD40L mediated SM fibroblast proliferation. Shown are bar graphs demonstrating SM fibroblast ^{3}H-thymidine incorporation following coculture in 1% FM with mitomycin-C treated CD40L$^-$ Jurkat B2.7 cells or CD40L$^+$ Jurkat B2.7 transfectants for 48 hours. Where indicated, SM fibroblasts were pretreated for 18 hours with rINF-γ (1000 U/ml) prior to the addition of mitomycin-C treated CD40L$^-$ B2.7 cells or CD40L$^+$ B2.7 transfectants. SM fibroblast proliferation was determined as outlined

in Materials and Methods for First Series of Experiments. Background proliferation of CD40L$^-$ Jurkat B2.7 cells and CD40L$^+$ Jurkat B2.7 transfectants was 185 ± 66 cpm and 65 ± 5 cpm, respectively. Background proliferation is subtracted in coculture experiments. Also shown are the proliferative responses of fibroblasts following culture in 1% FM or 10% FM. Similar results were obtained in 2 additional experiments. Error bars show observed error.

Figures 9A-D. Endothelial cells in skin express CD40 <u>in situ</u>. Shown are immunohistologic studies of frozen sections demonstrating the expression of: (a) CD40, skin (magnification 40x), (b) CD34, skin (magnification 40x), (c) CD21, skin (magnification 40x) and (d) control mouse IgG, skin (magnification 40x).

Figures 10A-D. Endothelial cells in muscle express CD40 <u>in situ</u>. Shown are immunohistologic studies of frozen sections demonstrating the expression of:(a) CD40, muscle (magnification 40x), (b) CD34, muscle (magnification 40x), (c) CD21, muscle (magnification 40x) and (d) control mouse IgG, muscle (magnification 40x).

Figure 11. Endothelial cells in spleen express CD40 <u>in situ</u>. Shown are immunohistologic studies of frozen sections demonstrating the expression of:(a) CD40, spleen (magnification 10x) and (b) control mouse IgG, spleen (magnification 10x).

Figure 12. Expression of CD40 on HUVEC cells <u>in vitro</u>. Shown are overlapping FACS analysis of CD14, CD40, CD45 or isotype control expression on HUVEC following the first passage. The mean fluorescence intensity of CD14, CD40, CD45 or isotype control expression is 7, 24, 5 and 9, respectively. Shown is representative of CD40 expression on HUVEC isolated from 15 umbilical cords.

Figure 13. Effect of CD40L-CD40 interactions on HUVEC CD54 (ICAM-1) expression. Shown are two-color contour graphs demonstrating the effects on HUVEC CD54 expression following culture with media, $CD40L^+$ Jurkat D1.1 cells or $CD40L^-$ Jurkat B2.7 cells for 6 hours. Where indicated, $CD40L^+$ D1.1 cells were pretreated with anti-CD40L mAb 5C8 or isotype control mAb P1.17. The X-axis demonstrates CD13 expression and the Y-axis demonstrates CD54 expression. The numbers in the upper right hand corner of each graph indicates percentage of $CD13^+$ cells expressing CD54 (background staining of control mAb is subtracted for each value). Shown is representative of 3 similar experiments with different HUVEC lines.

Figure 14. Effect of CD40L-CD40 interactions on HUVEC CD54 (ICAM-1), CD62E (E-selectin) and CD106 (VCAM-1) expression. Shown are bar graphs representing the percentage of HUVEC expressing CD54, CD62E or CD106 following culture for 6 hours with media, rIL-1α, $CD40L^+$ Jurkat D1.1 cells or $CD40L^-$ Jurkat B2.7 cells. Where indicated, $CD40L^+$ D1.1 cells were pretreated with anti-CD40L mAb 5C8 or isotype control mAb P1.17. HUVEC CD54, CD62E and CD106 expression was determined by two-color FACS analysis as shown in figure 3. Background staining of control mAb is subtracted for each value. Shown is representative of 3 similar experiments with different HUVEC lines.

Figure 15. Effect of CD40L expressing 293 kidney cell transfectants on HUVEC CD54, CD62E and CD106 expression. Shown are two-color contour graphs demonstrating the effects on HUVEC CD54, CD62E and CD106 expression following culture with media, $CD40L^+$ Jurkat D1.1 cells, $CD8^+$ 293 kidney cell transfectants or $CD40L^+$ 293 kidney cell transfectants for 6 hours. The X-axis demonstrates UEA-1 expression and the Y-axis

demonstrates CD54 (left panel), CD106 (middle panel) or CD62E (right panel) expression. The numbers in the upper right hand corner of each graph indicates the percentage of UEA-1^{+} cells expressing CD54, CD106 or CD62E, as indicated (background staining of control mAb is subtracted for each value). Shown is representative of 3 similar experiments with different HUVEC lines.

Figure 16A. Kinetic analysis of CD40L induced HUVEC CD54, CD62E and CD106 upregulation. Shown are the percentage of HUVEC expressing CD54, CD62E, or CD106 following culture with CD40L^{+} Jurkat D1.1 cells for 6 or 24 hours. The percentage of HUVEC expressing CD54, CD62E or CD106 was determined by two-color FACS analysis (background staining of control mAb is subtracted for each value). Shown is representative of 3 similar experiments with different HUVEC lines.

Figure 16B. Same as figure 16A except that HUVEC were cultured with CD40L - Jurkat B2.7 cells.

Figures 17A-Y: Atomic coordinates of crystal structure of soluble extracellular fragment of human CD40L containing residues Gly116-Leu261 (in Brookhaven Protein Data Bank format). (SEQ ID NO:1).

Detailed Description

This invention provides a method of inhibiting activation by CD40 ligand of cells bearing CD40 on the cell surface, comprising contacting the cells with an agent capable of inhibiting interaction between CD40 ligand and the cells, in an amount effective to inhibit activation of the cells. In one embodiment, the cells bearing CD40 on the cell surface are cells other than B cells. In another embodiment, they are plasma cells, including differentiated plasma cells such as myeloma cells.

This method may be used to inhibit activation of CD40-bearing cells either in vivo or ex vivo. "Interaction between CD40 ligand and CD40 on the cells" refers to one or more aspects, functional or structural, of a CD40-CD40 ligand interrelationship. Therefore, in one embodiment, an agent which inhibits interaction may competitively bind to CD40 ligand in such a way to block or diminish the binding of CD40 ligand to cellular CD40. In another embodiment an agent which inhibits interaction may associate with CD40 or CD40 ligand in a manner which does not inhibit binding of CD40 ligand to cellular CD40, but which influences the cellular response to the CD40 ligation, such as by altering the turnover rate of the cellular CD40 or the CD40-agent complex, by altering binding kinetics of CD40 with CD40 ligand, or by altering the rate or extent of cellular activation in response to CD40 ligation.

In specific embodiments of this invention, the non-B cell, CD40-bearing cells are fibroblasts, endothelial cells, epithelial cells, T cells, basophils, macrophages, Reed-Steinberg cells, or dendritic cells. In a more specific embodiment the epithelial cells are keratinocytes. In another embodiment, the macrophages

are foam cells (lipid-laden macrophages). Foam cells play a role in autoimmune diseases, for example rheumatoid arthritis and atherosclerosis.

In an embodiment of this invention the agent inhibits binding of CD40 ligand to CD40 on the cells.

In an embodiment of this method, the agent is a protein. In a more specific embodiment, the protein comprises an antibody or portion thereof, for example a Fab, $F(ab')_2$, complementarity determining region (CDR) light and/or heavy chain, antibody variable region light and/or heavy chain, or a portion thereof capable of specifically binding to CD40 ligand or CD40 ligand cell-surface receptor. The antibody can be a monoclonal or polyclonal antibody. In embodiments of this invention, the monoclonal antibody is a chimeric antibody, a humanized antibody, or a primatized antibody. In another embodiment the portion of the antibody comprises a single chain antibody. A single chain antibody is made up of variable regions linked by protein spacers in a single protein chain.

In an embodiment of the above-described method, the agent specifically binds to the antigen to which monoclonal antibody 5c8 specifically binds. In a specific embodiment, the agent is monoclonal antibody 5c8.

Monoclonal antibody 5c8 is produced by a hybridoma cell which was deposited on November 14, 1991 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The hybridoma was accorded ATCC Accession Number HB 10916.

In another embodiment, the antibody specifically binds to CD40. One example of an anti-CD40 antibody is the monoclonal mouse anti-human CD40, available from Genzyme Customer Service (Product 80-3702-01, Cambridge, MA). In other embodiments the monoclonal antibody is a chimeric antibody, a primatized antibody, a humanized antibody, or an antibody which includes a CDR region from a first human and an antibody scaffold from a second human.

In one embodiment of this invention the protein is soluble, monomeric CD40-L protein, comprising all or part of the extracellular region of CD40-L, or variant thereof. The extracellular region of CD40-L contains the domain that binds to CD40. Thus, soluble CD40-L can inhibit the interaction between CD40L and the CD40-bearing cell. This invention contemplates that sCD40-L may constitute the entire extracellular region of CD40-L, or a fragment or derivative containing the domain that binds to CD40.

The meaning of "chimeric", "primatized" and "humanized" antibody and methods of producing them are well known to those of skill in the art. See, for example, PCT International Publication No. WO 90/07861, published July 26, 1990 (Queen, et al.); and Queen, et al. Proc. Nat'l Acad. Sci.-USA (1989) 86: 10029). Methods of making primatized antibodies are disclosed, for example, in PCT International publication No. WO/02108, corresponding to International Application No. PCT/US92/06194 (Idec Pharmaceuticals); and in Newman, et al., Biotechnology (1992) 10:1455-1460, which are hereby incorporated by reference into this application.

Generally, a humanized antibody is an antibody comprising one or more complementarity determining regions (CDRs) of a non-human antibody functionally joined to human framework region segments. Additional residues

associated with the non-human antibody can optionally be present. Typically, at least one heavy chain or one light chain comprises non-human CDRs. Typically, the non-human CDRs are mouse CDRs. Generally, a primatized antibody is an antibody comprising one or more complementarity determining regions (CDRs) of an antibody of a species other than a non-human primate, functionally joined to framework region segments of a non-human primate. Additional residues associated with the species from which the CDR is derived can optionally be present. Typically, at least one heavy chain or one light chain comprises CDRs of the species which is not a nonhuman primate. Typically, the CDRs are human CDRs. Generally, a chimeric antibody is an antibody whose light and/or heavy chains contain regions from different species. For example one or more variable (V) region segments of one species may be joined to one or more constant (C) region segments of another species. Typically, a chimeric antibody contains variable region segments of a mouse joined to human constant region segments, although other mammalian species may be used.

In another embodiment of this invention, the protein is soluble CD40 protein (sCD40), comprising the extracellular region of CD40, or portion thereof, or variant thereof. sCD40 inhibits the interaction between CD40L and CD40-bearing cells. sCD40 may be in monomeric or oligomeric form.

Variants can differ from naturally occurring CD40 or CD40 ligand in amino acid sequence or in ways that do not involve sequence, or both. Variants in amino acid sequence are produced when one or more amino acids in naturally occurring CD40 or CD40 ligand is substituted with a different natural amino acid, an amino acid derivative or non-native amino acid. Particularly preferred variants include naturally occurring CD40 or

CD40 ligand, or biologically active fragments of naturally occurring CD40 or CD40 ligand, whose sequences differ from the wild type sequence by one or more conservative amino acid substitutions, which typically have minimal influence on the secondary structure and hydrophobic nature of the protein or peptide. Variants may also have sequences which differ by one or more non-conservative amino acid substitutions, deletions or insertions which do not abolish the CD40 or CD40 ligand biological activity. Conservative substitutions (substituents) typically include the substitution of one amino acid for another with similar characteristics such as substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. The non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Other conservative substitutions can be taken from Table 4, and yet others are described by Dayhoff in the Atlas of Protein Sequence and Structure (1988).

Table 4: Conservative Amino Acid Replacements

For Amino Acid	Code	Replace with any of
Alanine	A	D-Ala, Gly,beta-ALa, L-Cys,D-Cys
Arginine	R	D-Arg, Lys,homo-Arg, D-homo-Arg, Met,D-Met, Ile, D-Ile, Orn, D-Orn

Asparagine	N	D-Asn,Asp,D-Asp,Glu,D-Glu, Gln,D-Gln
Aspartic Acid	D	D-Asp,D-Asn,Asn, Glu,D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys,Met,D-Met,Thr, D-Thr
Glutamine	Q	D-Gln,Asn, D-Asn,Glu,D-Glu,Asp, D-Asp
Glutamic Acid	E	D-Glu,D-Asp,Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala,Pro, D-Pro, Beta-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Met, D-Met
Lysine	K	D-Lys,Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val, Norleu
Phenylalanine	F	D-Phe,Tyr, D-Thr,L-Dopa,His,D-His, Trp, D-Trp, Trans 3,4 or 5-phenylproline, cis 3,4 or 5 phenylproline
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D- or L-1-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O) D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr,Phe, D-Phe, L-Dopa, His,D-His

Valine	V	D-Val, Leu,D-Leu,Ile,D-Ile, Met, D-Met

Other variants within the invention are those with modifications which increase peptide stability. Such variants may contain, for example, one or more non-peptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are: variants that include residues other than naturally occurring L-amino acids, such as D-amino acids or non-naturally occurring or synthetic amino acids such as beta or gamma amino acids and cyclic variants. Incorporation of D- instead of L-amino acids into the polypeptide may increase its resistance to proteases. See, e.g., U.S. Patent 5,219,990.

The peptides of this invention may also be modified by various changes such as insertions, deletions and substitutions, either conservative or nonconservative where such changes might provide for certain advantages in their use.

In other embodiments, variants with amino acid substitutions which are less conservative may also result in desired derivatives, e.g., by causing changes in charge, conformation and other biological properties. Such substitutions would include for example, substitution of hydrophilic residue for a hydrophobic residue, substitution of a cysteine or proline for another residue, substitution of a residue having a small side chain for a residue having a bulky side chain or substitution of a residue having a net positive charge for a residue having a net negative charge. When the result of a given substitution cannot be predicted with certainty, the derivatives may be readily assayed according to the methods disclosed herein to determine the presence or absence of the desired characteristics.

Variants within the scope of the invention include proteins and peptides with amino acid sequences having at least eighty percent homology with the extracellular region of CD40 or the extracellular region of CD40 ligand. More preferably the sequence homology is at least ninety percent, or at least ninety-five percent.

Just as it is possible to replace substituents of the scaffold, it is also possible to substitute functional groups which decorate the scaffold with groups characterized by similar features. These substitutions will initially be conservative, i.e., the replacement group will have approximately the same size, shape, hydrophobicity and charge as the original group. Non-sequence modifications may include, for example, in vivo or in vitro chemical derivatization of portions of naturally occurring CD40 or CD40 ligand, as well as changes in acetylation, methylation, phosphorylation, carboxylation or glycosylation.

In a further embodiment the protein, including the extracellular region of CD40 ligand and CD40, is modified by chemical modifications in which activity is preserved. For example, the proteins may be amidated, sulfated, singly or multiply halogenated, alkylated, carboxylated, or phosphorylated. The protein may also be singly or multiply acylated, such as with an acetyl group, with a farnesyl moiety, or with a fatty acid, which may be saturated, monounsaturated or polyunsaturated. The fatty acid may also be singly or multiply fluorinated. The invention also includes methionine analogs of the protein, for example the methionine sulfone and methionine sulfoxide analogs. The invention also includes salts of the proteins, such as ammonium salts, including alkyl or aryl ammonium salts, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, thiosulfate, carbonate, bicarbonate, benzoate,

sulfonate, thiosulfonate, mesylate, ethyl sulfonate and benzensulfonate salts.

The soluble, monomeric CD40-L protein can comprise all or part of the extracellular region of CD40-L. The extracellular region of CD40-L contains the domain that binds to CD40. Thus, soluble CD40-L can inhibit the interaction between CD40L and the CD40-bearing cell. This invention contemplates that sCD40-L may constitute the entire extracellular region of CD40-L, or a fragment or derivative containing the domain that binds to CD40.

In another embodiment of this invention the protein comprising soluble extracellular region of CD40 or portion thereof further comprises an Fc region fused to the extracellular region of CD40 or portion thereof. In a specific embodiment the Fc region is capable of binding to protein A or protein G. In another embodiment the Fc region comprises IgG, IgG_1, IgG_2, IgG_3, IgG_4, IgA, IgA_1, IgA_2, IgM, IgD, or IgE.

In another embodiment of this invention, the sCD40 comprises CD40/Fc fusion protein. The fusion protein can be prepared using conventional techniques of enzymes cutting and ligation of fragments from desired sequences. Suitable Fc regions for the fusion protein are Fc regions that can bind to protein A or protein G, or that are capable of recognition by an antibody that can be used in purification or detection of a fusion protein comprising the Fc region. For example, the Fc region may include the Fc region of human IgG_1 or murine IgG_1. This invention also provides a nucleic acid molecule which encodes the CD40/Fc fusion protein.

The method of creating soluble forms of membrane molecules by recombinant means, in which sequences encoding the transmembrane and cytoplasmic domains are

deleted, is well known. See generally Hammonds et al., U.S. Patent No. 5,057,417. In addition, methods of preparing sCD40 and CD40/Fc fusion protein are well-known. See, e.g., PCT International Publication No. WO 93/08207; Fanslow et al., "Soluble Forms of CD40 Inhibit Biologic Responses of Human B Cells, "J. Immunol., vol. 149, pp.655-60 (July 1992).

In an embodiment of this invention, the agent is a small molecule. As used herein a small molecule is a compound having a molecular weight between 20 Da and $1x10^6$ Da, preferably from 50 Da to 2 kDa.

In an embodiment of this invention, the agent is selected by a screening method.

In a specific embodiment the small molecule or other agent is selected by a screening method which comprises, isolating a cell sample, for example a sample of a biological fluid (e.g., blood) from an animal; culturing the sample under conditions permitting activation of CD40-bearing cells contained therein; contacting the sample with an amount of cells expressing a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession no. HB 10916, or with a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession no. HB 10916, effective to activate the CD40-bearing cells; contacting the sample with an amount of a small molecule (or other pharmaceutical compound or agent) effective to inhibit activation of the CD40-bearing cells if the small molecule is capable of inhibiting activation of the CD40-bearing cells; and determining whether the cells expressing the protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession no. HB 10916, or with the protein which is specifically

recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession no. HB 10916 activate the CD40-bearing cells in the presence of the small molecule (or other pharmaceutical compound or agent). The cell sample may be isolated from diverse tissues, including cell lines in culture or cells isolated from an animal, such as dispersed cells from a solid tissue, cells derived from a bone marrow biopsy, or cells isolated from a body fluid such as blood or lymphatic fluid.

In another specific embodiment the agent (molecule) is selected based on a three-dimensional structure of soluble extracellular region of CD40 ligand or portion thereof capable of inhibiting interaction between CD40 ligand and CD40 on the cells. The agent may be selected from a library of known agents, modified from a known agent based on the three-dimensional structure, or designed and synthesized de novo based on the three-dimensional structure. In specific embodiments the agent (molecule) is designed by structure optimization of a lead inhibitory agent based on a three-dimensional structure of a complex of the soluble extracellular region of CD40 ligand or portion thereof with the lead inhibitory agent. A lead inhibitory agent is a molecule which has been identified which, when it is contacted with CD40 ligand or portion thereof, binds to and complexes with the soluble extracellular region of CD40 ligand, CD40, or portion thereof, thereby decreasing the ability of the complexed or bound CD40 ligand or CD40 ligand portion to activate CD40-bearing cells. In another embodiment, a lead inhibitory agent may act by interacting with either the extracellular region of CD40 ligand, CD40, or in a tertiary complex with both a portion of CD40 ligand and CD40, decreasing the ability of the complexed CD40 ligand-CD40 to activate the CD40-bearing cells. In the methods of the invention, the CD40 ligand may be either soluble or bound to cells such as

activated T cells, and may be either full length native CD40 ligand or portions thereof. Decreased ability to activate CD40-bearing cells may be measured in different ways. One way it may be measured is by showing that CD40 ligand, in the presence of inhibitor, causes a lesser degree of activation of CD40-bearing cells, as compared to treatment of the cells with a similar amount of CD40 ligand without inhibitor under similar conditions. Decreased ability to activate CD40-bearing cells may also be indicated by a higher concentration of inhibitor-CD40 ligand complex being required to produce a similar degree of activation of CD40-bearing cells under similar conditions, as compared to unbound CD40 ligand. At the extreme, the inhibitor-contacted CD40 ligand may be unable to activate CD40-bearing cells at concentrations and under conditions which allow activation of these cells by unbound CD40 ligand or a given portion thereof.

The agent (small molecule) can be selected by a computational screening method using the crystal structure of a soluble fragment of the extracellular domain of human CD40L containing residues Gly116-Leu261 (sCD40L(116-261)).

The crystal structure to be used with the screening method can be determined at 2 Å resolution by the method of molecular replacement. In brief, a soluble fragment of the extracellular domain of human CD40 ligand containing amino acid residues Gly 116 to the C-terminal residue Leu 261 are first produced in soluble form, then purified and crystallized. The crystals can be tested for diffraction capacity on the X-ray beam of an Elliot GX-13 generator. Molecular replacement and refinement can be done with the XPLOR program package and QUANTA (Molecular Simulations, Inc.) Software. In particular, a 3-dimensional model of human sCD40L can be constructed using the murine CD40L model using QUANTA protein

homology modeling software. This model can then be used as a probe for molecular replacement calculations and refined using XPLOR. This method of determining the crystal structure of sCD40L is described in more detail in Karpusas et al., "2 Å crystal structure of an extracellular fragment of human CD40 ligand," Structure (October 1995) 3(10):1031-1039. The atomic coordinates of sCD40L(116-261) are provided in Figures 17A-Y. The screening method for selecting an agent includes computational drug design and iterative structure optimization, as described below.

The agent may be a small molecule inhibitor selected using computational drug design. Using this method, the sCD40L crystal structure coordinates are used as an input for a computer program, such as DOCK, which outputs a list of small molecule structures that are expected to bind to CD40L. Use of such computer programs are well-known. See, e.g., Kuntz, "Structure-Based Strategies for drug design and discovery," Science, vol. 257, p. 1078 (1992). The list of small molecule structures can then be screened by biochemical assays for CD40L binding. Competition-type biochemical assays, which are well known, can be used. See, e.g., Bajorath et al., "Identification of residues of CD40 and its ligand which are critical for the receptor-ligand interaction," Biochemistry, 34, p. 1833 (1995). The structures that are found to bind to CD40L can thus be used as agents for the present invention. The agent may also be a modified small molecule, determined by interactive cycles of structure optimization. Using this approach, a small molecule inhibitor of CD40L found using the above computational approach or other approach can be co-crystallized with sCD40L and the crystal structure of the complex solved by molecular replacement. The information revealed through molecular replacement can be used to optimize the structure of the small molecule inhibitors

by clarifying how the molecules interact with CD40L. The small molecule may be modified to improve its physiochemical properties, including specificity and affinity for CD40L.

In an embodiment of this invention the agent specifically binds to CD40 on the cell surface. In a specific embodiment the agent is a protein, for example an antibody or the extracellular region of CD40 ligand. The antibody may be a polyclonal or monoclonal antibody. It is preferred that the monoclonal antibody be chimeric or humanized. It may also be primatized.

<u>In Vivo Use</u>

This invention provides a method of inhibiting activation by CD40 ligand of cells bearing CD40 on the cell surface, in a subject, comprising administering to the subject an agent capable of inhibiting interaction between CD40 ligand and the cells, in an amount effective to inhibit activation of the cells in the subject. In one embodiment, the cells bearing CD40 on the cell surface are cells other than B cells. In another embodiment, they are plasma cells, including differentiated plasma cells such as myeloma cells.

In specific embodiments of this invention, the non-B cell, CD40-bearing cells are fibroblasts, endothelial cells, epithelial cells, T cells, basophils, macrophages, Reed-Steinberg cells, or dendritic cells. In a more specific embodiment the epithelial cells are keratinocytes. In another embodiment, the macrophages are foam cells (lipid-laden macrophages). Foam cells play a role in autoimmune diseases, for example rheumatoid arthritis and atherosclerosis.

In an embodiment of this method, the agent is a protein.

In a more specific embodiment, the protein comprises an antibody or portion thereof, for example a Fab, $F(ab')_2$, complementarity determining region (CDR) light and/or heavy chain, antibody variable region light and/or heavy chain, or a portion thereof capable of specifically binding to CD40 ligand or CD40 ligand cell-surface receptor, or to CD40. One example of an anti-CD40 antibody is the monoclonal mouse anti-human CD40, available from Genzyme Customer Service (Product 80-3702-01, Cambridge, MA). The antibody can be a monoclonal or polyclonal antibody. In embodiments of this invention, the monoclonal antibody is a chimeric antibody, a humanized antibody, or a primatized antibody. In another embodiment the portion of the antibody comprises a single chain antibody. A single chain antibody is made up of variable regions linked by protein spacers in a single protein chain.

In an embodiment of the above-described method, the agent specifically binds to the antigen to which monoclonal antibody 5c8 (ATCC Accession No. HB 10916) specifically binds. In a specific embodiment, the agent is monoclonal antibody 5c8 (ATCC Accession No. HB 10916).

The compounds of this invention may be administered in any manner which is medically acceptable. This may include injections, by parenteral routes such as intravenous, intravascular, intraarterial, subcutaneous, intramuscular, intratumor, intraperitoneal, intraventricular, intraepidural, or others as well as oral, nasal, ophthalmic, rectal, topical, or inhaled. Sustained release administration is also specifically included in the invention, by such means as depot injections of erodible implants directly applied during surgery.

The compounds are administered at any dose per body

weight and any dosage frequency which is medically acceptable. For example, acceptable dosage for the compound of this invention (especially for the antibody or antibody portion of this invention) includes a range of between about 0.01 and 200 mg/kg subject body weight. A dosage range is between about 0.1 and 50 mg/kg. In a still more specific embodiment the dose is between about 1 and 30 mg/kg. The dosage is repeated at intervals ranging from each day to every other month. One dosing regimen is to administer a compound of the invention daily for the first three days of treatment, after which the compound is administered every 3 weeks, with each administration being intravenously at 5 or 10 mg/kg body weight.

Another regime is to administer a compound of the invention daily intravenously at 5 mg/kg body weight for the first three days of treatment, after which the compound is administered subcutaneously or intramuscularly every week at 10 mg per subject. Another regime is to administer a single dose of the compound of the invention parenterally at 20 mg/kg body weight, followed by administration of the compound subcutaneously or intramuscularly every week at 10 mg per subject.

The compounds of the invention may be administered as a single dosage for certain indications such as preventing immune response to an antigen to which a subject is exposed for a brief time, such as an exogenous antigen administered on a single day of treatment. Examples of such an antigen would include coadministration of a compound of the invention along with a gene therapy vector, or a therapeutic agent such as an antigenic pharmaceutical or a blood product. In indications where antigen is chronically present, such as in controlling immune reaction to transplanted tissue or to chronically administered antigenic pharmaceuticals, the compounds of

the invention are administered at intervals for as long a time as medically indicated, ranging from days or weeks to the life of the subject.

This invention provides a method of inhibiting an inflammatory response in a subject, comprising the above-described method of inhibiting activation by CD40 ligand of cells, other than B cells, bearing CD40 on the cell surface (e.g., fibroblast cells, endothelial cells, or keratinocyte cells) in a subject. Inflammatory responses are characterized by redness, swelling, heat and pain, as consequences of capillary dilation with edema and migration of phagocytic leukocytes. Inflammation is further defined by Gallin (Chapter 26, Fundamental Immunology, 2d ed., Raven Press, New York, 1989, pp. 721-733), which is hereby incorporated by reference.

This method is effective in inhibiting activation of any fibroblasts. In particular embodiments, the fibroblasts are synovial membrane fibroblasts, dermal fibroblasts, pulmonary fibroblasts, or liver fibroblasts. In particular embodiments, the condition dependent on CD40 ligand-induced activation of fibroblast cells is selected from the group consisting of arthritis, scleroderma, and fibrosis (e.g. fibrotic diseases of the liver and lung). In an embodiment of this invention, the fibrotic disease of the lung is caused by rheumatoid arthritis or scleroderma.

In an embodiment of this invention the arthritis is rheumatoid arthritis, non-rheumatoid inflammatory arthritis, arthritis associated with Lyme disease, or osteoarthritis. In another specific embodiment, the fibrosis is pulmonary fibrosis, hypersensitivity pulmonary fibrosis, or pneumoconiosis. In another specific embodiment, the fibrotic disease of the liver is Hepatitis-C, Hepatitis-B, Hepatitis non-B non-C,

cirrhosis, or cirrhosis of the liver secondary to a toxic insult, drugs, a viral infection, or an autoimmune disease. Alcohol consumption is one example of toxic insult which can cause cirrhosis of the liver. One example of a drug that can cause cirrhosis of the liver is Bleomycin. Others are known in the art.

Examples of viral infections which can cause fibrotic disease of the liver include, among others known to the art, Hepatitis B, Hepatitis C, and Hepatitis non-B non-C. Examples of autoimmune diseases which can cause fibrotic disease of the liver include, among others known to the art, primary biliary cirrhosis, and Lupoid hepatitis (autoimmune hepatitis). In specific embodiments the pulmonary fibrosis is pulmonary fibrosis secondary to adult respiratory distress syndrome (ARDS), drug-induced pulmonary fibrosis, idiopathic pulmonary fibrosis, or hypersensitivity pneumonitis; the pneumoconiosis is asbestosis, siliconsis, or Farmer's lung as well as other pneumoconioses that are known in the art to which this invention pertains.

This invention provides a method of treating a condition dependent on CD40 ligand-induced activation of endothelial cells in a subject, comprising the above-described method of inhibiting activation of endothelial cells by CD40 ligand in a subject.

In embodiments of this invention the condition dependent on CD40 ligand-induced activation of endothelial cells is selected from the group consisting of atherosclerosis, reperfusion injury, allograft rejection, organ rejection, and chronic inflammatory autoimmune diseases.

In a specific embodiment the atherosclerosis is accelerated atherosclerosis associated with organ transplantation. In situ CD40 and CD40L expression in

accelerated atherosclerosis associated with transplant rejection have been studied. Frozen sections of coronary arteries from 4 heart transplant patients that required retransplantation due to accelerated atherosclerosis were analyzed by routine immunohistochemistry utilizing anti-CD40 mAb G28.5, anti-CD40L mAb 5C8 or control mAbs. Routine H & E staining revealed the typical intimal hyperplasia, smooth muscle cell proliferation, and inflammatory cell infiltration associated with the disease. CD40 was widely expressed in the lesions: endothelial cells, foam cells and infiltrating inflammatory cells all express CD40. CD40L immunoreactivity was observed as discrete, faint staining of infiltrating mononuclear cells, presumably CD4+ T cells. Together, these studies demonstrate the presence of CD40L+ mononuclear cells and CD40+ endothelial cells, foam cells, and inflammatory cells in situ in lesions of accelerated atherosclerosis associated with transplantation.

In another specific embodiment the chronic inflammatory autoimmune disease is vasculitis, rheumatoid arthritis, scleroderma, or multiple sclerosis.

This invention provides a method of treating a condition dependent on CD40 ligand-induced activation of keratinocytes in a subject, comprising the above-described method of inhibiting activation of keratinocyte cells by CD40 ligand in a subject.

In a specific embodiment the condition dependent on CD40 ligand-induced activation of keratinocytes is psoriasis.

This invention provides a method of treating a condition dependent on CD40 ligand-induced activation of macrophages in a subject, comprising the above-described method of inhibiting activation of macrophages by CD40

ligand in a subject. In specific embodiments, the condition dependent on CD40 ligand-induced activation of macrophages is atherosclerosis or rheumatoid arthritis.

The subject which can be treated by the above-described methods is an animal. Preferably the animal is a mammal. Examples of mammals which may be treated include, but are not limited to, humans; rodents such as the murine animals rats and mice, as well as rabbits, and guinea pig; cow; horse; sheep; goat; pig; dog and cat.

This invention also provides a method of treating a condition dependent on CD40 ligand-induced activation of plasma cells in a subject (including malignant plasma cells), comprising administering to the subject an agent capable of inhibiting interaction between CD40 ligand and the cells, in an amount effective to inhibit activation of the cells in the subject. Plasma cells are differentiated B cells. In a specific embodiment the condition is multiple myeloma.

This invention provides a method of promoting the growth of cells bearing CD40 on the cell, comprising contacting the cells with an amount of CD40 ligand effective to promote growth of the cells. In an embodiment the cells are cells bearing CD40 on the cell surface other than B cells. In specific embodiments the non-B cells bearing CD40 on the cell surface are endothelial cells, fibroblasts, epithelial cells, T cells, or basophils. In another embodiment the cells are plasma cells, including differentiated plasma cells such as myeloma cells.

This invention further provides a pharmaceutical composition comprising a therapeutically effective amount of the agent described herein capable of inhibiting interaction between CD40 ligand and cells bearing CD40 on the cell surface, and a pharmaceutically acceptable

carrier.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

FIRST SERIES OF EXPERIMENTS

Materials and Methods

Patients Studied

All RA patients studied met the American College of Rheumatology criteria for RA (19). The diagnosis of OA was established by the patients' physicians utilizing clinical and radiographic criteria. One patient with chronic inflammatory arthritis (IA) of unknown etiology was also studied.

Monoclonal antibodies and T cell lines

The IgG2a murine anti-CD40L mAb (5C8) was previously generated (3). Hybridomas anti-MHC Class I (W6/32), anti-MHC Class II (L243), anti-CD14 (3C10), anti-CD40 (G28.5) and anti-CD45 (GAP 8.3) were purchased from American Type Culture Collection (ATCC) (Rockville, MD). Hybridoma ascites was purified on a Protein G column (Pharmacia, Piscataway, NJ). Anti-CD13 and anti-CD54 mAbs were purchased from Biosource International (Camarillo, CA). Anti-CD106 mAb was kindly provided by Biogen (Cambridge, MA) and biotinylated as previously described (20). Isotype control mAbs utilized for FACS analysis were purchased from Becton-Dickinson (San Jose, CA) or Caltag (South San Francisco, CA). P1.17 is a control IgG2a murine mAb obtained from Biogen and utilized for functional studies.

D1.1 is a Jurkat T cell subclone that constitutively expresses CD40L (3, 21). B2.7 is a $CD40L^-$ Jurkat subclone (3, 21). $CD40L^+$ Jurkat B2.7 transfectants expressing full length CD40L protein were generated as previously reported (20).

Isolation of fibroblasts

Synovial membrane was obtained from 6 RA or 8 OA patients undergoing joint replacement surgery. SM from one patient with IA was collected at arthroscopy. SM was cut into small pieces and cultured in 100 mm tissue culture petri dishes (Corning, Corning, NY) or 25 cm^2 flasks (Costar, Cambridge, MA) with Isocove's Modified Dulbecco's Media (Gibco, Grand Island, NY) supplemented with 10% FCS (Summit Biotechnology, Ft. Collins, CO) and 1% penicillin-streptomycin (Sigma, St. Louis, MO) (10% FM). Synoviocytes were allowed to adhere for several days at which time tissue debris and non-adherent cells were removed. Synoviocytes were grown to confluence and passaged by treatment with 1% trypsin-EDTA (Sigma). Synoviocytes were studied between 1-6 passages <u>in vitro</u>. A normal dermal fibroblast line frozen following the second passage (CCD 965SK) was purchased from ATCC. Dermal fibroblast lines were studied between 2-4 passages.

Studies on the effects of cytokines on fibroblast CD40 expression

To study the effects of cytokines on fibroblast CD40 expression, cells were cultured in 6 well plates (Nunc, Denmark) and grown to near confluence. The media was aspirated and fibroblasts then cultured with the indicated concentrations of rINF-γ (Biogen), rIL-1α (R & D, Minneapolis, MN), rTNF-α (Upstate Biotechnology, Lake Placid, NY), rIL-4 (Biosource International), rGM-CSF (Immunex, Seattle, WA) or combinations of cytokines in 3 ml of 10% FM. At the indicated time points, the media was aspirated, the cells washed once with saline and 1 ml of 1% trypsin-EDTA added to the wells. After 7 minutes cold 10% FM was added to the wells and the cells collected for FACS analysis.

Studies on functional consequences of fibroblast CD40 ligation.

To determine the effect of CD40 ligation on the expression of fibroblast cell surface molecules, fibroblasts were cultured in 6 well plates as described above. When the fibroblasts were near confluence 1 x 10^6 $CD40L^+$ Jurkat D1.1 cells, $CD40L^-$ Jurkat B2.7 cells or $CD40L^+$ Jurkat B2.7 transfectants were added to the culture. Where indicated, D1.1 cells were pretreated with anti-CD40L mAb 5C8 (10 μg/ml) or isotype control mAb P1.17 (10 μg/ml) prior to the addition to fibroblasts. After 24 hours the cells were collected by trypsinization and two-color FACS analyses performed.

For studies determining the effect of CD40 ligation on fibroblast proliferation, approximately 5 x 10^3 cells were added to flat bottom 96 well plates (Nunc) in 10% FM. After 18 hours the media was changed to 1% FM and rINF-γ 1000 U/ml added to the indicated cells. After an additional 18 hours, 1 x 10^5 mitomycin-C (Sigma) treated $CD40L^+$ Jurkat B2.7 transfectants or CD40L Jurkat B2.7 cells in 1% FM were added to the fibroblasts. Anti-CD40L mAb 5C8 (5 μg/ml) or control mAb P1.17 (5 μg/ml) were also added to some wells as indicated. 10% FM was added to some cells as a control for the induction of SM fibroblast proliferation. Cultures were maintained for an additional 48 hours and pulsed with 1 μCi ^{3}H thymidine for the last 18 hours of the experiment. Following trypsinization, ^{3}H thymidine incorporation was determined by harvesting onto glass fiber filter strips (Cambridge Technologies, Watertown, MA) and scintillation counting (BetaCounter, Pharmacia).

To determine the effect of CD40 ligation on IL-6 production, a bioassay utilizing the IL-6 responsive murine B cell line B9 was performed (22). Equal numbers of fibroblasts in 10% FM were seeded in 96 well plates as

mentioned above. After adhering overnight, 1×10^5 mitocycin-C treated $CD40L^+$ Jurkat D1.1 cells, CD40L Jurkat B2.7 cells or $CD40L^+$ Jurkat B2.7 transfectants were added to the fibroblasts. Where indicated, D1.1 cells were pretreated with anti-CD40L mAb 5C8 (10 μg/ml) or control mAb P1.17 (10 μg/ml). Control wells consisted of Jurkat cells cultured alone. After 48 hours, serial dilutions of fibroblast or control supernatants or rIL-6 were added to 7.5×10^3 B9 cells in 96 well plates. B9 cells were maintained in culture for 96 hours, pulsed with 1 μCi ^3H thymidine for the last 18 hours and harvested as mentioned above.

Cytofluorographic analysis

The methods utilized for cytofluorographic analysis have been previously described (21). In all experiments the cells were first treated with aggregated human immunoglobulin (Enzyme International, Fallbrook, CA) to block non-specific Ig binding. For single-color FACS analysis, cells were stained with saturating concentrations of primary antibody for 30-60 minutes at 4° C. Following washing, FITC conjugated $F(ab)_2$ goat anti-mouse IgG (Cappel, Cochranville, PA) was added for 30-60 minutes at 4° C. The cells were washed and fixed with 1% formaldehyde prior to FACS analysis. For two-color FACS analysis, cells were simultaneously stained with the indicated FITC or PE conjugated mAbs for 30-60 minutes at 4° C. Fluorescence intensity was measured on a FACScan cytofluorograph with the Consort-30 software (Becton-Dickinson, Mountainview, CA). Mean fluorescence intensity (MFI) refers to values normalized to the log scale as calculated by Becton-Dickinson C30 software.

Results

Expression of CD40 on cultured SM or dermal fibroblasts.

To determine whether SM fibroblasts express CD40, SM

derived from 6 RA, 1 IA, or 8 OA patients was first minced and placed in culture after which non-adherent cells were discarded. As expected, primary cultures of adherent cells were pleiomorphic with regard to morphology and phenotype. A minority of cells assumed a stellate morphology or a rounded appearance characteristic of macrophages. However, the majority of cells in primary culture had fibroblast-like morphology and phenotype, i.e., $CD45^{-}CD14^{-}$MHC Class II^{-} (figure 1). Virtually all cells had fibroblast-like morphology and phenotype following 2-3 passages in vitro.

Five RA fibroblast lines were studied for CD40 expression following the first or second passage in vitro and were $CD40^{+}$ by FACS analysis (figure 1). An IA fibroblast line similarly expresses CD40 (table 1). One RA fibroblast line had been in culture for 2 months prior to analysis and was $CD40^{-}$ (data not shown). Eight OA fibroblast lines were studied for CD40 expression following the first or second passage in vitro and all were $CD40^{+}$ (figure 1). To determine if fibroblast CD40 expression was restricted to SM fibroblasts, normal dermal fibroblasts were analyzed for CD40 expression following 2-4 passages in vitro. To variable degrees, all 3 dermal fibroblast lines studied also express cell surface CD40 molecules (figure 2). However, CD40 expression on synovial membrane or dermal fibroblasts decreased with increasing time in culture such that some fibroblast lines became $CD40^{-}$ after 3-4 passages (data not shown). These studies demonstrate that dermal fibroblasts or SM fibroblasts isolated from patients with various arthritides can express CD40 in vitro.

Effect of cytokines on fibroblast CD40 expression

Interferon-γ (INF-γ) is known to upregulate CD40 expression on B cells (23), macrophages (12) and thymic epithelial cells (15). Moreover, IL-1α or TNF-α upregulates CD40 expression on thymic epithelial cells (15). Therefore, it was next asked if rINF-γ, rIL-1α or rTNF-α regulates CD40 expression on cultured SM fibroblasts. Cells were cultured with the indicated cytokines and CD40 expression determined by FACS analysis. As a control for the effects of these cytokines on the expression of SM fibroblast cell surface molecules, CD54 (ICAM-1) expression was also determined (24). rINF-γ upregulates SM fibroblast CD40 expression (table 1 and figure 3). In contrast, rIL-1α and rTNF-α have minimal effect on SM fibroblast CD40 expression (table 1 and figure 3). However, either rIL-1α or rTNF-α augment the effect of rINF-γ on SM fibroblast CD40 expression (figure 3). rINF-γ also induces CD40 expression on SM fibroblasts that had lost CD40 expression during serial passages in culture (data not shown). Moreover, rINF-γ upregulates CD40 expression on dermal fibroblasts (figure 2). rIL-4 or rGM-CSF upregulate CD40 expression on B cells (25) or monocytes (12), respectively. However, rIL-4 or rGM-CSF have no effect on SM fibroblast CD40 expression (data not shown). Together, these studies demonstrate that rINF-γ induces and upregulates fibroblast CD40 expression and the addition of rIL-1α or rTNF-α augments this effect.

Effect of CD40L-CD40 interactions on SM fibroblast CD54 (ICAM-1) and CD106 (VCAM-1) expression

Because CD40 triggering is known to upregulate a variety of cell surface molecules on B cells, including adhesion molecules (26), it was determined if CD40 ligation upregulates CD54 or CD106 expression on SM fibroblasts. SM fibroblasts were cultured with CD40L⁺ Jurkat D1.1 cells in the presence or absence of anti-

CD40L mAb 5C8 or control mAb. SM fibroblasts were also cultured with $CD40L^{-}$ Jurkat B2.7 cells or $CD40L^{+}$ Jurkat B2.7 transfectants. After the indicated period of time in culture, SM fibroblast CD54 or CD106 expression was determined by two-color FACS analysis. CD13 expression was utilized to discriminate SM fibroblasts from Jurkat T cells (27). $CD40L^{+}$ D1.1 cells, but not control $CD40L^{-}$ B2.7 cells, induce a 2-4 fold increase in SM fibroblast CD54 expression (figures 4 and 5) in a manner that is specifically inhibited by mAb 5C8 but not by control mAb (figure 4). Moreover, $CD40L^{+}$ D1.1 and $CD40L^{+}$ Jurkat B2.7 transfectants, but not control $CD40L^{-}$ B2.7 cells, similarly upregulate SM fibroblast CD106 expression (figure 5). Together, these results demonstrate that CD40L-CD40 interactions upregulate SM fibroblast CD54 and CD106 expression.

Effect of CD40 ligation on SM fibroblast IL-6 secretion. Ligation of CD40 induces B cells (28) and monocytes (12) to produce IL-6. Interestingly, SM fibroblasts produce IL-6 <u>in vivo</u> (29, 30) and <u>in vitro</u> (31). The next series of experiments asked if CD40L-CD40 interactions effect IL-6 secretion by SM fibroblasts. Therefore, SM fibroblasts were cultured with mitomycin-C treated $CD40L^{+}$ Jurkat D1.1 cells in the presence or absence of anti-CD40L mAb 5C8 or control mAb. Additionally, SM fibroblasts were cultured with $CD40L^{-}$ Jurkat B2.7 cells or $CD40L^{+}$ Jurkat B2.7 transfectants. Fibroblast supernatants or control supernatants from Jurkat cells cultured alone were collected after 48 hours and dilutions added to the IL-6 responsive murine B cell line B9. D1.1 cells and $CD40L^{+}$ B2.7 transfectants, but not CD40L B2.7 cells, augment SM fibroblast IL-6 secretion (figure 6). Additionally, anti-CD40L mAb 5C8, but not control mAb, inhibits this effect of D1.1 cells. Control supernatants collected from Jurkat cells cultured alone did not induce B9 proliferation (See description of Figure 6). These

studies indicate that ligation of CD40 on SM fibroblasts augments IL-6 secretion.

Effect of CD40L-CD40 interactions on SM fibroblast proliferation

Because CD40 ligation induces B cell proliferation (5, 21), it was next asked if CD40L$^+$ cells induce proliferation of SM fibroblasts. Therefore, SM fibroblasts were cultured overnight in 1% FM to arrest growth, as previously described (32), and further additions to the cells were performed in 1% FM, unless otherwise indicated. Mitomycin-C treated CD40L$^+$ B2.7 transfectants or CD40L$^-$ B2.7 cells were than added to the SM fibroblasts. Where indicated, co-culture experiments also included anti-CD40L mAb 5C8 or isotype control mAb P1.17. In some experiments, SM fibroblasts were pretreated overnight with rINF-γ prior to the addition of CD40L$^+$ B2.7 transfectants. Because fibroblasts are known to proliferate in the presence of media containing 10% FCS ((32)), each experiment included control fibroblasts cultured in 10% FM. ^{3}H thymidine incorporation was determined after 48 hours. CD40L$^+$ B2.7 transfectants, in contrast to parental CD40L$^-$ B2.7 cells, induce SM fibroblast proliferation (figure 7). Furthermore, anti-CD40L mAb 5C8 specifically inhibits the ability of CD40L$^+$ B2.7 transfectants to induce fibroblast proliferation (figure 7). In addition, pretreatment of SM fibroblasts with rINF-γ augments the capacity of CD40L$^+$ B2.7 transfectants to induce SM fibroblast proliferation (figure 8). Together, these data demonstrate that CD40L mediated signals induce SM fibroblast proliferation <u>in vitro</u> and this effect is enhanced by rINF-γ.

Discussion

This study extends current knowledge of CD40 expression and function by specifically demonstrating that: 1)

cultured SM or dermal fibroblasts express cell surface CD40 molecules as determined by FACS analysis, 2) rINF-γ upregulates fibroblast CD40 expression and this effect is augmented by rIL-1α or rTNF-α, 3) CD40L-CD40 interactions upregulates SM fibroblast CD54 and CD106 expression, 4) ligation of CD40 augments SM fibroblast IL-6 production and 5) induces SM fibroblast proliferation. Together, these data demonstrate that CD40L-CD40 interactions functionally activate fibroblasts in vitro.

Several lines of evidence suggest that T cells modulate fibroblast functions in vivo. This is of importance because fibroblasts play reparative roles following tissue injury by producing extracellular matrix proteins. In addition, lymphocytes, macrophages and fibroblasts are the predominant cell types in granulomatous inflammatory reactions characteristic of certain infections. Moreover, T cells directly or indirectly mediate fibroblast activation and collagen deposition seen in diseases such as scleroderma or chronic graft versus host disease (33-35).

Animal models demonstrate that T cells modulate fibroblast function during host responses to tissue injury. In this regard, studies of wound healing show that wound strength and hydroxyproline content are significantly decreased by treating mice with cyclosporine A (36) or T cell depleting anti-Thy 1.2 mAb (37). T cells also modulate outcome in various animal models of fibrosis. For example, bleomycin-induced pulmonary fibrosis is significantly attenuated in athymic mice relative to control euthymic mice (38). Moreover, joint or liver inflammatory reactions and collagen deposition are also significantly reduced in athymic rats following intraperitoneal injection of streptococcal cell wall extracts (39, 40).

One study suggests that human fibroblasts can express CD40 in vivo. Potocnik and coworkers studied the expression and distribution of various cell surface molecules, including CD40, on RA PBL, SF and SM (18). By immunohistochemistry they noted CD40 expression on a variety of cells in RA SM, including cells with spindle shape morphology suggestive of fibroblasts. SM fibroblasts are a predominant cellular component of the rheumatoid pannus. By producing collagenase, PGE2, IL-6 and other mediators, synovial fibroblasts are thought to be important contributors to the joint destruction characteristic of RA (30, 41-43). While electron microscopic studies have demonstrated direct T-fibroblast contact in rheumatoid synovial membrane (44), most studies have suggested that macrophage derived cytokines, such as IL-1 or TNF-α, activate fibroblasts (30). These studies suggest that direct contact mediated by CD40L-CD40 interactions also provides activation and proliferative signals to SM fibroblasts.

The mechanism by which CD40L mediated signals augment SM fibroblast proliferation is currently unknown. It is possible that CD40L-CD40 interactions induce the secretion of cytokines, such as IL-1, GM-CSF and FGF, which can stimulate SM fibroblast proliferation in an autocrine or paracrine manner (31). CD40 ligation also induces B cells to express c-myc (45) a proto-oncogene associated with proliferating cells. Immunohistologic studies demonstrate that RA SM fibroblast-like synoviocytes express c-myc in situ (46). Therefore, it will be of interest to specifically determine if CD40 ligation also induces c-myc expression in SM fibroblasts.

Similar to CD40 ligation on B cells (26), CD40L-CD40 interactions augment expression of fibroblast CD54 expression. In addition, CD40L-CD40 interactions upregulate fibroblast CD106 expression. CD54 and CD106

play key role in recruiting immune cells to sites of inflammation by interacting with CD11a/CD18 (LFA-1) or CD49d (VLA-4), respectively, expressed on leukocytes (24). There is also evidence that these ligand-counterligand interactions enhance proliferative signals to T cells (47). CD54 and CD106 are known to be expressed on RA fibroblast-like synoviocytes in vivo ((48-50)) and various cytokines upregulate synovial fibroblast CD54 and CD106 expression in vitro (49, 51, 52). Moreover, T cell adhesion to SM fibroblasts in vitro is partly mediated by CD11a/CD18-CD54 interactions (53) and CD49d-CD106 interactions (49). Therefore, CD54 and CD106 upregulation on SM fibroblasts by CD40L$^+$ T cells may represent a mechanism to augment cytokine mediated inflammatory cell recruitment/retainment to SM. Additionally, CD40L mediated SM fibroblast CD54 and CD106 upregulation may play direct signaling roles to T cells via interactions with their counter-receptors.

It is of interest that in vivo administration of a hamster anti-murine CD40L mAb (MR1) prevents the induction of collagen-induced arthritis, a murine model of RA (54). The fact that MR1 blocks the production of anti-collagen autoantibodies likely relates to the known role of CD40L-CD40 interactions in T cell dependent humoral immune responses (9-11). Moreover, MR1 prevents the development of synovial lining cell thickening and SM inflammatory cell infiltration characteristic of collagen-induce arthritis (54). These studies suggest that T cell-fibroblast CD40L-CD40 interactions play roles in mediating inflammatory reactions seen in collagen-induced arthritis, an also plays immunopathogenic roles in human fibrotic diseases such as RA or scleroderma, mediated in part by T cell-dependent fibroblast activation. Moreover, this study provides new rational for blocking CD40L-CD40 interactions as therapy for human diseases mediated by CD4$^+$ T cell induced fibroblast

activation.

TABLE 1

	OA.2		OA.3		IA.1	
Stimuli	CD40	CD54	CD40	CD54	CD40	CD54
Media	18	129	76	134	47	120
rINF-γ	56	703	228	668	95	755
rIL-1α	22	286	82	304	37	292
rTNF-α	22	568	96	506	66	594

Table 1 Legend. Cytokine regulation of SM fibroblast CD40 expression. Shown is CD40 expression (mean fluorescence intensity) as determined by FACS analysis on the indicated SM fibroblast lines following coculture with media, rINF-γ (1000 U/ml), rIL-1α (10 pg/ml) or rTNF-α (200 U/ml). Background staining (MFI) of a control mAb is subtracted for each value.

SECOND SERIES OF EXPERIMENTS

Materials and Methods

Monoclonal antibodies, lectins and T cell lines

The IgG2a murine anti-CD40L mAb (5C8) was previously generated (20). Hybridomas W6/32 (anti-MHC Class I), L243 (anti-MHC Class II), 3C10 (anti-CD14), THB.5 (anti-CD21), G28.5 (anti-CD40) and GAP 8.3 (anti-CD45) were purchased from American Type Culture Collection (ATCC) (Rockville, MD). Hybridoma ascites was purified on a Protein G column (Pharmacia, Piscataway, NJ). FITC conjugated anti-CD13, FITC conjugated anti-CD19 and PE conjugated anti-CD54 mAbs was purchased from Biosource International (Camarillo, CA) and anti-CD34 mAb was obtained from Biogenex (San Ramon, CA). An additional anti-CD54 mAb, as well as anti-CD62E and anti-CD106 mAbs, were kindly provided by Biogen (Cambridge, MA). L243 and mAbs provided by Biogen were biotinylated as previously described (37). PE conjugated anti-CD80 and biotinylated anti-CD86 mAbs were purchased from Becton Dickinson (San Jose, CA) and PharMingen (San Diego, CA), respectively. Isotype control mAbs utilized for FACS analysis were purchased from Becton Dickinson or Caltag Laboratories (South San Francisco, CA). P1.17 is an irrelevant control IgG2a murine mAb (Biogen) utilized for functional studies. FITC conjugated UEA-1 were obtained from Sigma (St. Louis, MO).

D1.1 is a Jurkat T cell subclone that constitutively expresses CD40L (20, 42). B2.7 is a $CD40L^-$ Jurkat T cell subclone (20, 42). Stably transfected $CD40L^+$ 293 kidney cells or $CD8^+$ 293 kidney cells were generated as previously reported (37). Ramos 2G6 B cells respond to CD40L mediated signals (38, 39) and were obtained from ATCC.

Endothelial cell cultures

Human umbilical vein endothelial cells (HUVEC) were isolated as previously reported (40, 41). HUVEC were cultured in M199 media (Gibco, Grand Island, NY) supplemented with 25% FCS (Summit Biotechnology, St. Collins, CO), 5% human serum (Gemini, Calabasas, CA), heparin 90 μg/ml (Sigma), endothelial cell growth factor 15 μg/ml (Collaborative Research, Bedford, MA) and 1% penicillin-streptomycin (Sigma) (M199 complete media). HUVEC were passaged by treatment for 3 minutes with 1% Trypsin-EDTA (Sigma). All HUVEC experiments were performed in M199 complete media following 1-3 passages.

Studies on the effects of cytokines on HUVEC CD40 expression

To study the effects of cytokines on CD40 expression, HUVEC were cultured in 6 well plates (Nunc, Denmark) and grown to near confluence. The media was aspirated and HUVEC were then incubated with rIFN-γ 1000 U/ml (Biogen), rIL-1α 10 pg/ml (R & D, Minneapolis, MN) or rTNF-α 200 U/ml (Upstate Biotechnology, Lake Placid, NY) in 3 ml of M199 complete media. At the indicated times, media was aspirated, cells were washed once with saline and 1 ml of 1% trypsin-EDTA was added to the wells. Cold Isocove's Modified Dulbecco's Media (Gibco) containing 10% FCS (Summit) was added to the wells after 3 minutes and the cells collected for FACS analysis.

Studies on functional consequences of HUVEC CD40 ligation.

To study the effect of CD40 ligation on the expression of HUVEC cell surface molecules, cells were cultured in 6 well plates as described above. When HUVEC were near confluence 1×10^6 $CD40L^+$ Jurkat D1.1 cells, $CD40L^-$ Jurkat B2.7 cells, $CD40L^+$ 293 kidney cell transfectants or CD8 kidney cell transfectants were added to the culture. Where indicated, $CD40L^+$ cells were pretreated with anti-CD40L mAb 5C8 (10

μg/ml) or isotype control mAb P1.17 (10 μg/ml) prior to the addition to HUVEC. After the indicated time in culture the cells were collected by trypsinization and two-color FACS analyses performed.

Functional studies of CD40 ligation on Ramos 2G6 cells. Control experiments of CD40 ligation on Ramos 2G6 cells were performed by culturing 2 x 10^5 Ramos 2G6 cells with 1 x 10^5 D1.1 cells or control cells for 24h hours in 96 well plates containing 200 μl of Isocove's Modified Dulbecco's Media (Gibco) containing 10% FCS (Summit) and 1% penicillin-streptomycin (Sigma).

Cytofluorographic analysis

The methods utilized for cytofluorographic analysis have been previously described (20, 42). In all experiments the cells were first treated with aggregated human immunoglobulin (Enzyme International, Fallbrook, CA) to block non-specific Ig binding. For single-color FACS analysis, cells were stained with saturating concentrations of primary antibody for 30-60 minutes at 4°C. Following washing, FITC conjugated $F(ab)_2$ goat anti-mouse IgG (Jackson Immunoresearch Laboratories, West Grove, PA) was added for 30-60 minutes at 4° C. The cells were washed and fixed with 1% formaldehyde prior to FACS analysis. For two-color FACS analysis, cells were first stained with the indicated biotinylated mAbs. Following washing, cells were then stained with streptavidin-PE (Calbiochem, La Jolla, CA) and FITC conjugated anti-CD13 mAb or FITC conjugated UEA-1, as indicated. HUVEC were distinguished from Jurkat cells in two-color FACS analysis by positive staining with anti-CD13 mAb or UEA-1, a lectin that selectively binds endothelial cells (43). Fluorescence intensity was measured on a FACScan cytofluorograph with the Consort-30 software (Becton-Dickinson, Mountainview, CA). Mean fluorescence

intensity (MFI) refers to values normalized to the log scale as calculated by the Consort 30 software.

Characterization of endothelial cell CD40 expression in situ.

Frozen sections of normal spleen, thyroid, skin, muscle, kidney, lung or umbilical cord were studied for CD40 expression, as previously described (38). Immunohistologic analysis was performed with the indicated mAbs and reactivity detected using Vector ABC Elite kit and 3-amino-9-ethylcarbazole (AEC) (Vector Laboratories, Burlingame, CA) according to manufacture's instructions. Control frozen sections were stained with appropriate concentrations of mouse IgG (Sigma).

Results

In situ and in vitro characterization of endothelial cell CD40 expresssion.

The first series of experiments were performed to determine if normal endothelial cells express CD40 in situ. Therefore, frozen sections obtained from normal spleen, thyroid, skin, muscle, kidney, lung or umbilical cord were stained with anti-CD40 mAb or control mouse IgG and endothelial cell reactivity noted. Additional controls included staining with anti-CD34 mAb (reactive with hematopoietic stem cells and endothelial cells (44)) or anti-CD21 mAb (reactive with B cell cells and epithelial cells (17)). Endothelial cells from all tissues studied express CD40 in situ. Figures 9-11 demonstrate representative CD40 staining of endothelial cells in normal skin (figure 9), muscle (figure 10) and spleen (figure 11). The pattern of endothelial reactivity was similar to that seen with anti-CD34 mAb (figures 9 and 10). In contrast, endothelial cells did not react with anti-CD21 mAb (figures 9 and 10) or mouse IgG (figures 9-11).

To further explore endothelial cell CD40 expression and function <u>in vitro</u> it was next asked if cultured human umbilical vein endothelial cells (HUVEC) also express CD40. HUVEC were isolated, grown to confluence and CD40 expression determined by FACS analysis following trypsinization. The cells morphologically resembled endothelial cells and phenotypic analysis demonstrated that the cells were $CD13^+$ and reactive with UEA-1, a lectin that selectively binds endothelial cells (43). In addition, the cells were $CD14^-$ $CD45^-$MHC Class II^- by FACS analysis. Therefore, these cultures did not contain significant numbers of contaminating non-endothelial cells. HUVEC constitutively express CD40 <u>in vitro</u> (figure 12). Similar results were obtained from HUVEC isolated from 15 individuals.

To determine if pro-inflammatory cytokines regulate endothelial cell CD40 expression, as has been shown for B cells (45), monocytes (14), thymic epithelial cells (18) and fibroblasts (19), HUVEC were cultured with rIFN-γ, rIL-1α, or rTNF-α for 48 hours. rINF-γ, in contrast to rIL-1α or rTNF-α, induces 2-3 fold increase in HUVEC CD40 expression (table 2). Together, these studies demonstrate that endothelial cells from normal tissue express CD40 <u>in situ</u> and <u>in vitro</u> and that rIFN-γ upregulates endothelial cell CD40 expression <u>in vitro</u>.

Effect of CD40L-CD40 interactions on HUVEC CD54, CD62E and CD106 expression.

Activated endothelial cells express cell surface molecules, such as CD54, CD62E and CD106 that play important roles in mediating intercellular adhesive interactions (1, 2). Interestingly, ligation of CD40 on B cells (46) or fibroblasts (19) induces the upregulation of adhesion molecules. Therefore, it was next asked if CD40L-CD40 interactions effect the expression of CD54, CD62E or CD106

expression on HUVEC in vitro as determined by two-color FACS analysis. HUVEC were cultured with CD40L+ Jurkat D1.1 cells or CD40L- Jurkat B2.7 cells. Where indicated, Jurkat D1.1 cells were pretreated with anti-CD40L mAb 5C8 or control mAb prior to the addition to HUVEC. As a positive control, HUVEC were also cultured with rIL-1α. CD40L+ Jurkat D1.1 cells, but not CD40L- Jurkat B2.7 cells, induce CD54, CD62E and CD106 upregulation on HUVEC (figures 13 and 14). This effect of D1.1 cells is inhibited by anti-CD40L mAb 5C8 but not by an isotype control mAb (figures 13 and 14). These studies strongly suggest that CD40L-CD40 interactions upregulate CD54, CD62E and CD106 expression on HUVEC.

Effect of CD40L+ 293 kidney cell transfectants on HUVEC CD54, CD62E and CD106 expression.

To determine if CD40L mediated signals were sufficient, in the absence of additional lymphoid specific interactions, to upregulate endothelial cell adhesion molecules, HUVEC were cultured with stably transfected CD40L+ 293 kidney cells or control CD8+ 293 transfectants. As a positive control, HUVEC were also cultured with CD40L+ D1.1 cells. Similar to CD40L+ D1.1 cells, CD40L+ 293 kidney cell transfectants upregulate CD54, CD62E and CD106 expression on HUVEC (figure 15). Control 293 CD8 transfectants have no effect on HUVEC CD54, CD62E or CD106 expression. Together, these studies demonstrate that CD40L-CD40 interactions are sufficient to upregulate these adhesion molecules on HUVEC in vitro.

Analysis of the kinetics of CD40L mediated HUVEC CD54, CD62E and CD106 upregulation.

The kinetics of CD54, CD62E or CD106 upregulation by rIL-1α or rTNF-α in vitro has been well established (1, 2). CD54 and CD106 are upregulated 6 hours following activation and expression persist for greater than 24 hours. In contrast, CD62E expression peaks 6 hours following activation and

returns to baseline (no expression) by 24 hours. In the next series of experiments the kinetics of CD40L induced HUVEC CD54, CD62E or CD106 upregulation were determined. HUVEC were cultured with CD40L$^+$ D1.1 cells or CD40L B2.7 cells and analyzed at various time points for CD54, CD62E or CD106 expression. Following culture with CD40L$^+$ D1.1 cells, HUVEC CD54 or CD106 expression was upregulated by 6 hours and persisted in expression for greater than 24 hours (figure 16). In contrast, CD40L induced CD62E expression peaked by 6 hours and returned to baseline by 24 hours (figure 16). Therefore, the kinetics of CD40L, rTNF-α or rIL-1α mediated upregulation of HUVEC CD54, CD62E or CD106 are similar.

Determining if CD40L-CD40 interactions upregulate CD80, CD86 or MHC Class II expression on HUVEC.

Activated endothelial cells are competent to express MHC Class II molecules and deliver costimulatory signals to T cells (10, 47-49). Ligation of CD40 on B cells or dendritic cells upregulates MHC Class II expression, as well as, the expression of the costimulatory molecules CD80 and CD86 (36, 37, 50-52). Therefore the next series of experiments determined if CD40L-CD40 interactions similarly upregulates MHC Class II, CD80 or CD86 expression on HUVEC. HUVEC were cultured with CD40L$^+$ D1.1 cells or CD40L$^-$ B2.7 cells for 24 or 48 hours and CD80, CD86 and MHC Class II expression determined by two-color FACS analysis. As a positive control for the effect of HUVEC CD40 ligation, CD54 expression was also determined. In addition, HUVEC were also cultured with rIFN-γ as a control for MHC Class II upregulation. As a positive control for CD40L mediated CD80, CD86 and MHC Class II upregulation, D1.1 cells were cultured with Ramos 2G6 B cells (38-39). In contrast to the effects of CD40 ligation on B cells or dendritic cells, CD40L-CD40 interactions do not upregulate MHC Class II, CD80

or CD86 expression on HUVEC (table 3).

Discussion

CD40 is a cell surface molecule constitutively expressed on a variety of cells, including B cells (12, 13), monocytes (14), dendritic cells (15), epithelial cells (17, 18), basophils (16) and fibroblasts (19). The counter-receptor for CD40 is CD40L, a 30-33 kDa activation-induced, transiently expressed $CD4^+$ T cell surface molecule (20-25). It is shown that endothelial cells in spleen, thyroid, skin, muscle, kidney, lung or umbilical cord express CD40 _in situ_. This finding is consistent with a previous report that endothelial cells in rheumatoid arthritis synovial membrane express CD40 (11). In addition, human umbilical vein endothelial cells (HUVEC) express CD40 _in vitro_. Most importantly, CD40 expression on endothelial cells is functionally significant because $CD40L^+$ Jurkat T cells or $CD40L^+$ 293 kidney cell transfectants, but not control cells, upregulate the expression of intercellular adhesion molecules CD54 (ICAM-1), CD62E (E-selectin) and CD106 (VCAM-1) on HUVEC. The results disclosed herein demonstrate that endothelial cells express CD40 and CD40L-CD40 interactions induce endothelial cell activation _in vitro_.

Endothelial cells play central roles in inflammatory responses in part by expressing CD54, CD62E and CD106 (1, 2). These adhesion molecules interact with specific cell surface receptors on leukocytes and promote the transmigration of inflammatory cells across the endothelial cell barrier. The expression of these particular endothelial cell surface molecules are tightly regulated (1, 2). Resting endothelial cells express low levels of CD54 and minimal or no CD62E or CD106. However, endothelial cells upregulate CD54, CD62E and CD106 expression following activation with IL-1 or TNF. These findings demonstrate a

means by which activated $CD4^+$ T cells upregulate endothelial cell adhesion molecules by direct cell-cell contact.

Because CD40L expression is also tightly regulated, it is likely that CD40L-CD40 interactions occur during Ag driven immune responses. In this regard, in vitro studies demonstrate that resting $CD4^+$ T cells do not express detectable CD40L (20-22, 25, 53). However, CD40L is transiently expressed on activated $CD4^+$ T cells in vitro; peak expression is seen 6 hours following activation and levels return to baseline (no expression) by 24-48 hours (20, 21, 53). CD40L is also rapidly down-modulated by CD40 expressing cells in a process that is at least partly due to receptor-mediated endocytosis (54). In vivo, CD40L expression is normally restricted to $CD4^+$ T cells in secondary lymphoid tissue (38), the site of MHC restricted, Ag specific T-B interactions. However, immunohistologic studies of rheumatoid arthritis synovial membrane or psoriatic plaques demonstrates the presence of $CD40L^+CD4^+$ T cells. These studies suggest that APCs at sites of inflammation induce infiltrating $CD4^+$ T cell to express CD40L. $CD40L^+CD4^+$ T cells then play roles in augmenting the inflammatory process by interacting with $CD40^+$ endothelial cells. The functional consequences of this interaction enable further adhesion and transmigration of immune cells at sites of inflammation.

The fact that CD40 ligation regulates the expression of endothelial cell surface adhesion molecules is consistent with a general role for CD40 signalling in regulating the expression and/or function of adhesion molecules on a variety of cells. In this regard, it has been shown that CD40L mediated signals induce CD54 and CD106 upregulation on fibroblasts cultured from synovial membrane (19). CD40 ligation also upregulates CD54 expression on B cells (46)

and induces CD54 dependent homoaggregation of B cells (55). Interestingly, pretreatment of B cells with anti-CD40 mAb augments heterotypic interactions of B cells with activated endothelial cells _in vitro_ in a manner dependent on CD49d (VLA-4)/CD106 interactions (56). Because CD40 ligation did not upregulate B cell CD49d expression, it was hypothesized that CD40 mediated signals induced CD49d activation.

CD40 ligation on B cells or dendritic cells also upregulates expression of MHC Class II, as well as, the costimulatory molecules CD80 and CD86 (36, 37, 50-52). Interestingly, endothelial cells stimulated with rIFN-γ are competent to express MHC Class II _in vitro_ (57) and endothelial cells _in situ_ within inflammatory tissue can express MHC Class II (10, 58-60). Moreover, endothelial cells are competent to present Ag to T cells _in vitro_ and deliver appropriate costimulatory signals to T cells required for IL-2 production and proliferation (10, 47-49).

However, it is shown here that CD40L-CD40 interactions do not upregulate MHC Class II, CD80 or CD86 expression on HUVEC _in vitro_. This finding is consistent with previous studies suggesting that human endothelial cells do not express CD80 (47, 61). The costimulatory molecules expressed on endothelial cells are not precisely known. Work by Pober and colleagues demonstrate that blocking CD2-CD54 (LFA-3) interactions inhibits the ability of endothelial cells to induce allogenic T cell proliferation (47, 48). However, it is unclear if CD2-CD58 interactions enhance intercellular adhesiveness and/or deliver costimulatory signals to T cells. It will be of interest to determine if CD40L mediated signals modulate the capacity of endothelial cells to activate T cells.

Finally, endothelial cells are activated in a variety of

diseases mediated by CD4$^+$ T cells. For example, endothelial cell surface adhesion molecules are upregulated in rheumatoid arthritis (62), scleroderma (63) and in transplant rejection (64). In addition, CD4$^+$ T cells play roles in atherosclerosis (65) and accelerated atherosclerosis associated with transplantation (60). The precise mechanistic role of CD40L mediated interactions with endothelial cells in these diseases is not known. However, an antibody to CD40L, MR1, inhibits murine models of diseases mediated by CD4$^+$ T cells and/or inflammatory cell infiltrates. For example, MR1 prevents the synovial lining cell hypertrophy and cellular infiltrate associated with collagen-induce arthritis, a murine model of rheumatoid arthritis (66). Moreover, MR1 inhibits a murine model of multiple sclerosis (EAE) and inhibits allograft rejection (67). Blocking CD40L dependent interactions with endothelial cells and/or fibroblasts mediates, in part, these effects of MR1. The results disclosed herein suggest that CD40L-CD40 interactions on the surface of endothelial cells play immunopathogenic roles in inflammatory diseases.

TABLE 2

	HUVEC Expression	
Stimuli	CD40 (MFI)	CD54 (MFI)
Media	17	22
rINF-γ	42	44
rIL-1α	24	51
rTNF-α	22	54

Table 2 Legend. Effect of cytokines on HUVEC CD40 expression. Shown is the mean fluorescence intensity (MFI) of CD40 or CD54 expression on HUVEC cultured in the presence or absence of rIFN-γ (1000 U/ml), rIL-1α (10 pg/ml) or rTNF-α (200 U/ml) for 48 hours. CD40 or CD54 MFI was determined by FACS analysis and background staining of control mAb is subtracted for each value. Similar results were obtained in 2 additional experiments with different HUVEC lines.

TABLE 3

	HUVEC Expression (MFI)				Ramos Expression (MFI)			
Conditions	CD54	CD80	CD86	MHC II	CD54	CD80	CD86	MHC II
Media	8	0	1	0	22	0	7	128
D1.1	78	0	0	0	71	8	13	223
B2.7	23	0	1	1	25	1	7	127
rIFN-γ	16	0	0	97	ND	ND	ND	ND

Table 3 Legend. Effect of CD40L-CD40 interactions on HUVEC MHC Class II, CD80 and CD86 expression. Shown is the mean fluorescence intensity of HUVEC CD54, CD80, CD86 or MHC Class II expression following culture with media, rIFN-γ (1000 U/ml), CD40L$^+$ Jurkat D1.1 cells or CD40L B2.7 cells for 48 hours. In a parallel experiment, the CD40L responsive Ramos 2G6 B cell line (38-39) was cultured with media, CD40L$^+$ Jurkat D1.1 cells or CD40L B2.7 cells for 24 hours. HUVEC or Ramos 2G6 MHC Class II, CD54, CD80 and CD86 expresssion was determined by two-color FACS analysis. Background staining of control mAb is subtracted for each value. Shown is representative of 3 similar experiments with different HUVEC lines. ND= not done.

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SEQUENCE LISTING

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Thomas, David W.

(ii) TITLE OF INVENTION: THERAPEUTIC APPLICATIONS FOR THE ANTI-T-BAM (CD40-L) MONOCLONAL ANTIBODY 5c8

(iii) NUMBER OF SEQUENCES: 1

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(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: Not Yet Known
(B) FILING DATE: Herewith
(C) CLASSIFICATION:

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(A) APPLICATION NUMBER: US 08/566,258
(B) FILING DATE: 01-DEC-1995
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(C) CLASSIFICATION

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 146 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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15                  20                  25

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Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile
        45                  50                  55

Tyr Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser
            60                  65                  70

Gln Ala Pro Phe Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly
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Arg Phe Glu Arg Ile Leu Leu Arg Ala Ala Asn Thr His Ser
85                  90                  95

Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His Leu Gly Gly
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Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn Val
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Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser
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Phe Gly Leu Leu Lys Leu
                145
```

What is claimed is:

1. A method of inhibiting activation by CD40 ligand of cells bearing CD40 on the cell surface, other than B cells, comprising contacting the cells with an agent capable of inhibiting interaction between CD40 ligand and the cells, in an amount effective to inhibit activation of the cells.

2. The method of claim 1, wherein the CD40-bearing cells are selected from the group consisting of fibroblasts, endothelial cells, epithelial cells, T cells, basophils, macrophages, Reed-Steinberg cells, and dendritic cells.

3. The method of claim 2, wherein the epithelial cells are keratinocytes.

4. The method of claim 1, wherein the agent inhibits binding of CD40 ligand to CD40 on the cells.

5. The method of claim 1, wherein the agent is a protein.

6. The method of claim 5, wherein the protein comprises an antibody or portion thereof.

7. The method of claim 6, wherein the antibody is a monoclonal antibody.

8. The method of claim 7, wherein the monoclonal antibody is a chimeric antibody.

9. The method of claim 7, wherein the monoclonal antibody is a humanized antibody.

10. The method of claim 7, wherein the monoclonal antibody is a primatized antibody.

11. The method of claim 6, wherein the portion of the antibody comprises a complementarity determining region or variable region of a light or heavy chain.

12. The method of claim 6, wherein the portion of the antibody comprises a complementarity determining region or a variable region.

13. The method of claim 12, wherein the portion of the antibody comprises a Fab, or a single chain antibody.

14. The method of claim 5, wherein the protein comprises soluble extracellular region of CD40 ligand, or variants thereof including conservative substituents, or portion thereof; or soluble extracellular region of CD40, or variants thereof including conservative substituents, or portion thereof.

15. The method of claim 14, wherein the soluble extracellular region of CD40 ligand or CD40 is a monomer.

16. The method of claim 14, wherein the soluble extracellular region of CD40 is an oligomer.

17. The method of claim 14, wherein the protein comprising soluble extracellular region of CD40 or portion thereof further comprises an Fc region fused to the extracellular region of CD40 or portion thereof.

18. The method of claim 17, wherein the Fc region is

capable of binding to protein A or protein G.

19. The method of claim 17, wherein the Fc region comprises IgG, IgA, IgM, IgD, or IgE, or subclasses thereof.

20 The method of claim 19, wherein:
the IgG is IgG_1, IgG_2, IgG_3, or IgG_4; or
the IgA is IgA_1 or IgA_2.

21. The method of claim 1, wherein the agent specifically binds to the antigen to which monoclonal antibody 5c8 (ATCC Accession No. HB 10916) specifically binds.

22. The method of claim 21, wherein the agent is an antibody.

23. The method of claim 22, wherein the antibody is monoclonal antibody 5c8 (ATCC Accession No. HB 10916).

24. The method of claim 1, wherein the agent is a small molecule.

25. The method of claim 1, wherein the agent specifically binds to CD40 on the cell surface.

26. The method of claim 25, wherein the agent is a protein.

27. The method of claim 26, wherein the protein is an antibody.

28. The method of claim 27, wherein the antibody is a monoclonal antibody.

29. The method of claim 28, wherein the monoclonal antibody is chimeric, humanized, or primatized.

30. The method of claim 26, wherein the protein comprises the extracellular region of CD40 ligand.

31. The method of claim 1, wherein the agent is nonprotein.

32. The method of claim 1, wherein the agent is selected from a library of known agents.

33. The method of claim 1, wherein the agent is modified from a known agent.

34. The method of claim 33, wherein the modified agent is designed by structure optimization of a lead inhibitory agent based on a three-dimensional structure of a complex of soluble extracellular region of CD40 ligand or portion thereof with the lead inhibitory agent.

35. The method of claim 1, wherein the agent is selected by a screening method, which comprises:

isolating a sample of cells;

culturing the sample under conditions permitting activation of CD40-bearing cells;

contacting the sample with cells expressing a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, or with a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, effective to

activate the CD40-bearing cells;

contacting the sample with an amount of the agent effective to inhibit activation of the CD40-bearing cells if the agent is capable of inhibiting activation of the CD40-bearing cells; and

determining whether the cells expressing the protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, or with the protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, activate the CD40-bearing cells in the presence of the agent.

36. The method of claim 35, wherein the agent is selected from a library of known agents.

37. The method of claim 36, wherein the known agents are nonprotein agents.

38. A method of inhibiting activation by CD40 ligand of cells bearing CD40 on the cell surface, other than B cells, in a subject, comprising administering to the subject an agent capable of inhibiting interaction between CD40 ligand and the cells, in an amount effective to inhibit activation of the cells in the subject.

39. The method of claim 38, wherein the CD40-bearing cells are selected from the group consisting of fibroblasts, endothelial cells, epithelial cells, T cells, basophils, macrophages, Reed-Steinberg cells, and dendritic cells.

40. The method of claim 39, wherein the epithelial cells are keratinocytes.

41. The method of claim 38, wherein the agent inhibits binding of CD40 ligand to CD40 on the cells.

42. The method of claim 38, wherein the agent is a protein.

43. The method of claim 42, wherein the protein comprises an antibody or portion thereof.

44. The method of claim 43, wherein the antibody is a monoclonal antibody.

45. The method of claim 43, wherein the monoclonal antibody is a chimeric antibody.

46. The method of claim 44, wherein the monoclonal antibody is a humanized antibody.

47. The method of claim 44, wherein the monoclonal antibody is a primatized antibody.

48. The method of claim 43, wherein the portion of the antibody comprises a complementarity determining region or variable region of a light or heavy chain.

49. The method of claim 43, wherein the portion of the antibody comprises a complementarity determining region or a variable region.

50. The method of claim 49, wherein the portion of the antibody comprises a Fab, or a single chain antibody.

51. The method of claim 38, wherein the agent

specifically binds to the antigen to which monoclonal antibody 5c8 (ATCC Accession No. HB 10916) specifically binds.

52 The method of claim 51, wherein the agent is an antibody.

53. The method of claim 52, wherein the antibody is monoclonal antibody 5c8 (ATCC Accession No. HB 10916).

54. The method of claim 38, wherein the subject is a mammal.

55. The method of claim 54, wherein the mammalian subject is a human.

56. The method of claim 54, wherein the mammalian subject is a rodent.

57. The method of claim 38, wherein the protein comprises soluble extracellular region of CD40 ligand, or variants thereof including conservative substituents, or portion thereof; or soluble extracellular region of CD40, or variants thereof including conservative substituents, or portion thereof.

58. The method of claim 57, wherein the soluble extracellular region of CD40 ligand or CD40 is a monomer.

59. The method of claim 57, wherein the soluble extracellular region of CD40 is an oligomer.

60. The method of claim 57, wherein the protein comprising soluble extracellular region of CD40 or

portion thereof further comprises an Fc region fused to the extracellular region of CD40 or portion thereof.

61. The method of claim 60, wherein the Fc region is capable of binding to protein A or protein G.

62. The method of claim 60, wherein the Fc region comprises IgG, IgA, IgM, IgD, or IgE, or subclasses thereof.

63. The method of claim 62, wherein:
the IgG is IgG_1, IgG_2, IgG_3, or IgG_4; or
the IgA is IgA_1 or IgA_2.

64. The method of claim 38, wherein the agent is a small molecule.

65. The method of claim 38, wherein the agent specifically binds to CD40 on the cell surface.

66. The method of claim 65, wherein the agent is a protein.

67. The method of claim 66, wherein the protein is an antibody.

68. The method of claim 67, wherein the antibody is a monoclonal antibody.

69. The method of claim 68, wherein the monoclonal antibody is chimeric, humanized, or primatized.

70. The method of claim 66, wherein the protein comprises the extracellular region of CD40 ligand.

71. The method of claim 38, wherein the agent is

nonprotein.

72. The method of claim 38, wherein the agent is selected from a library of known agents.

73. The method of claim 38, wherein the agent is modified from a known agent.

74. The method of claim 73 wherein the modified agent is designed by structure optimization of a lead inhibitory agent based on a three-dimensional structure of a complex of soluble extracellular region of CD40 ligand or portion thereof with the lead inhibitory agent.

75. The method of claim 38, wherein the agent is selected by a screening method, which comprises:

isolating a sample of cells;

culturing the sample under conditions permitting activation of CD40-bearing cells;

contacting the sample with cells expressing a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, or with a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, effective to activate the CD40-bearing cells;

contacting the sample with an amount of the agent effective to inhibit activation of the CD40-bearing cells if the agent is capable of inhibiting activation of the CD40-bearing cells; and

determining whether the cells expressing the protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, or with the protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, activate the CD40-bearing cells in the presence of the agent.

76. The method of claim 75, wherein the agent is selected from a library of known agents.

77. The method of claim 76, wherein the known agents are nonprotein agents.

78. A method of inhibiting an inflammatory response in a subject, comprising the method of claim 38.

79. A method of treating a condition dependent on CD40 ligand-induced activation of fibroblast cells in a subject, comprising the method of claim 38.

80. The method of claim 79, wherein the fibroblasts are synovial membrane fibroblasts, dermal fibroblasts, pulmonary fibroblasts, or liver fibroblasts.

81. The method of claim 79, wherein the condition is selected from the group consisting of arthritis, scleroderma, and fibrosis.

82. The method of claim 81, wherein the arthritis is rheumatoid arthritis, non-rheumatoid inflammatory arthritis, arthritis associated with Lyme disease, or osteoarthritis.

83. The method of claim 81, wherein the fibrosis is

pulmonary fibrosis, hypersensitivity pulmonary fibrosis, or a pneumoconiosis.

84. The method of claim 83, wherein the pulmonary fibrosis is pulmonary fibrosis secondary to adult respiratory distress syndrome, drug-induced pulmonary fibrosis, idiopathic pulmonary fibrosis, or hypersensitivity pneumonitis.

85. The method of claim 83, wherein the pneumoconiosis is asbestosis, siliconosis, or Farmer's lung.

86. The method of claim 81, wherein the fibrosis is a fibrotic disease of the liver or lung.

87. The method of claim 86, wherein the fibrotic disease of the lung is caused by rheumatoid arthritis or scleroderma.

88. The method of claim 86, wherein the fibrotic disease of the liver is selected from the group consisting of:

Hepatitis-C;
Hepatitis-B;
cirrhosis;
cirrhosis of the liver secondary to a toxic insult;
cirrhosis of the liver secondary to drugs;
cirrhosis of the liver secondary to a viral infection; and
cirrhosis of the liver secondary to an autoimmune disease.

89. The method of claim 88, wherein the toxic insult is alcohol consumption.

90. The method of claim 88, wherein the viral infection

is Hepatitis B, Hepatitis C, or hepatitis non-B non-C.

91. The method of claim 88, wherein the autoimmune disease is primary biliary cirrhosis, or Lupoid hepatitis.

92. A method of treating a condition dependent on CD40 ligand-induced activation of endothelial cells in a subject, comprising the method of claim 38.

93. The method of claim 92, wherein the condition is selected from the group consisting of atherosclerosis, reperfusion injury, allograft rejection, organ rejection, and chronic inflammatory autoimmune diseases.

94. The method of claim 93, wherein the atherosclerosis is accelerated atherosclerosis associated with organ transplantation.

95. The method of claim 93, wherein the chronic inflammatory autoimmune disease is vasculitis, rheumatoid arthritis, scleroderma, or multiple sclerosis.

96. A method of treating a condition dependent on CD40 ligand-induced activation of epithelial cells in a subject, comprising the method of claim 38.

97. The method of claim 96 wherein the epithelial cells are keratinocytes, and the condition is psoriasis.

98. A method of inhibiting activation by CD40 ligand of myeloma cells bearing CD40 on the cell surface, comprising contacting the cells with an agent capable of inhibiting interaction between CD40

ligand and the cells, in an amount effective to inhibit activation of the cells.

99. A method of inhibiting activation by CD40 ligand of myeloma cells bearing CD40 on the cell surface, in a subject, comprising administering to the subject an agent capable of inhibiting interaction between CD40 ligand and the cells, in an amount effective to inhibit activation of the cells in the subject.

100. A method of treating a condition dependent on CD40 ligand-induced activation of myeloma cells in a subject, comprising the method of inhibiting activation by CD40 ligand of myeloma cells bearing CD40 on the cell surface of claim 99.

101. The method of claim 100, wherein the condition is multiple myeloma.

FIGURE 1A

RA

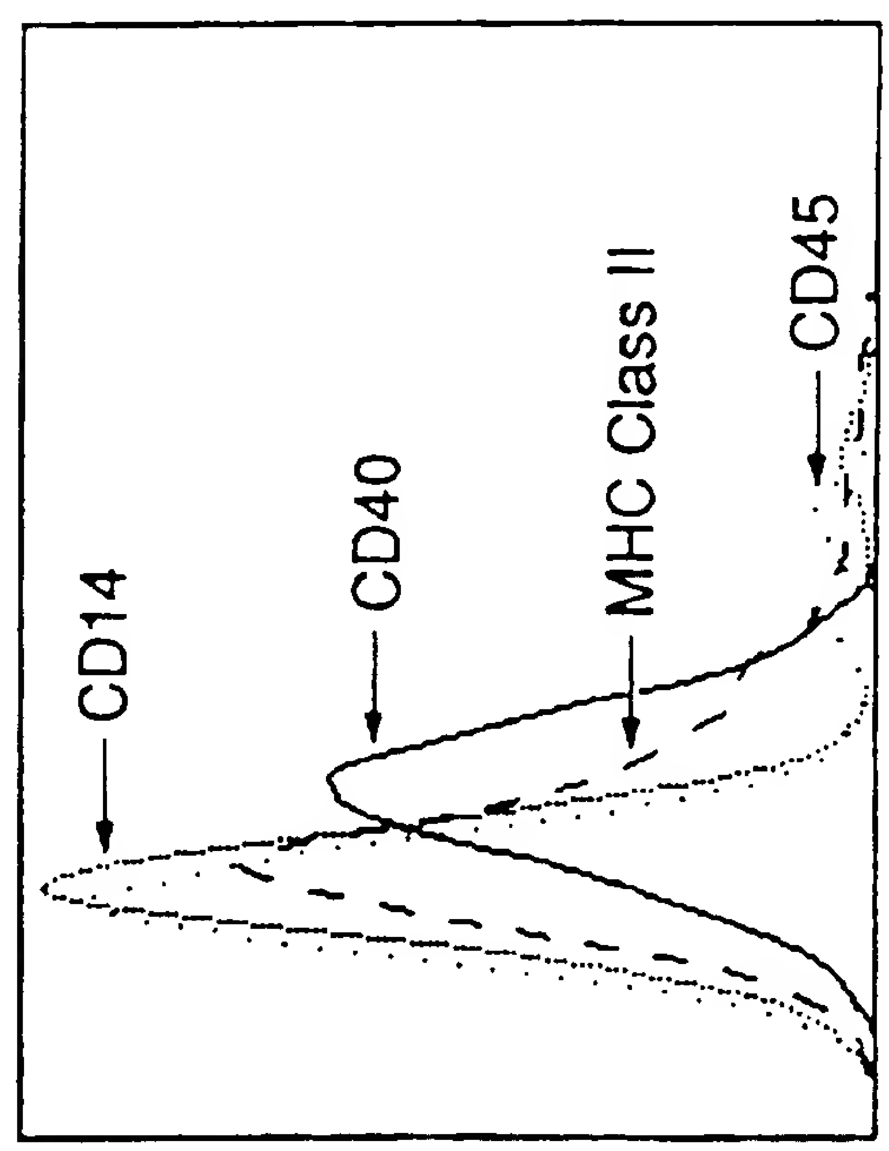


FIGURE 1B

OA

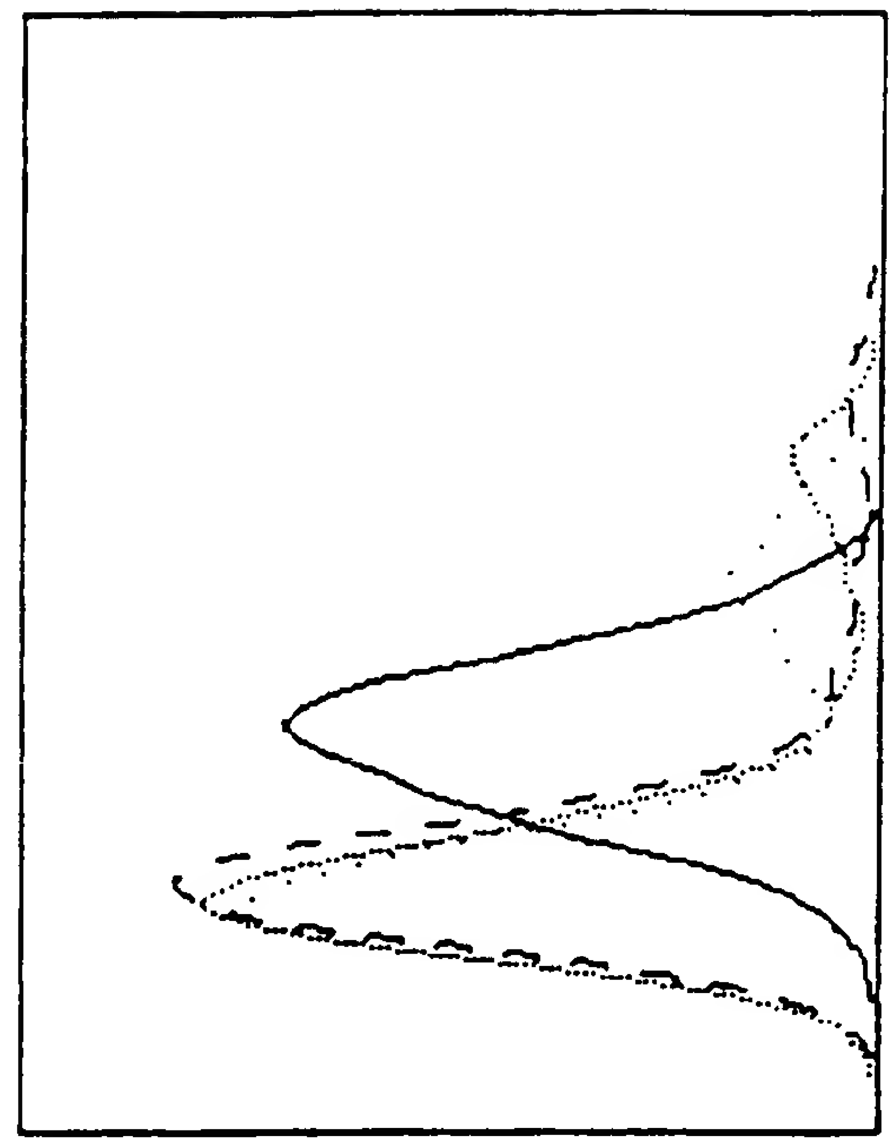


FIGURE 2A

Resting

CCD
965 SK

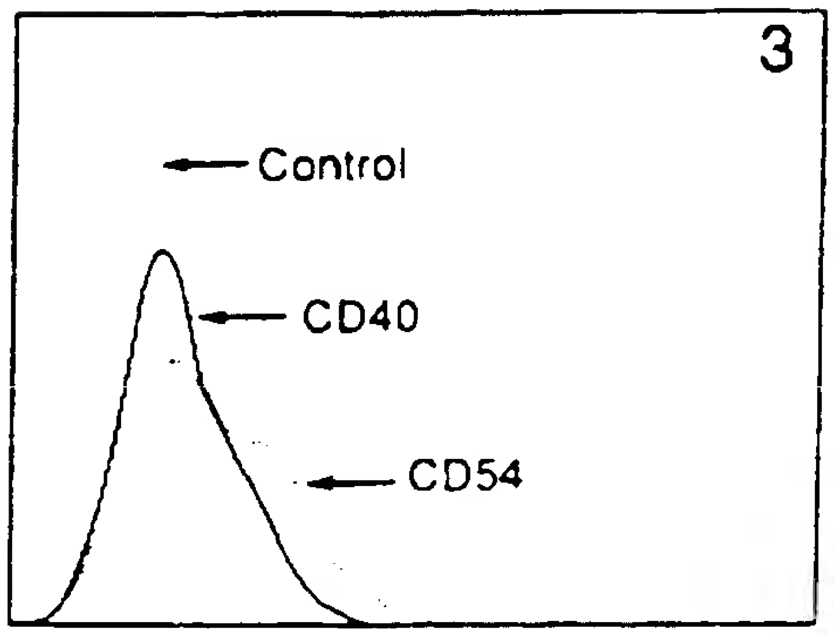


FIGURE 2B

γ - INF

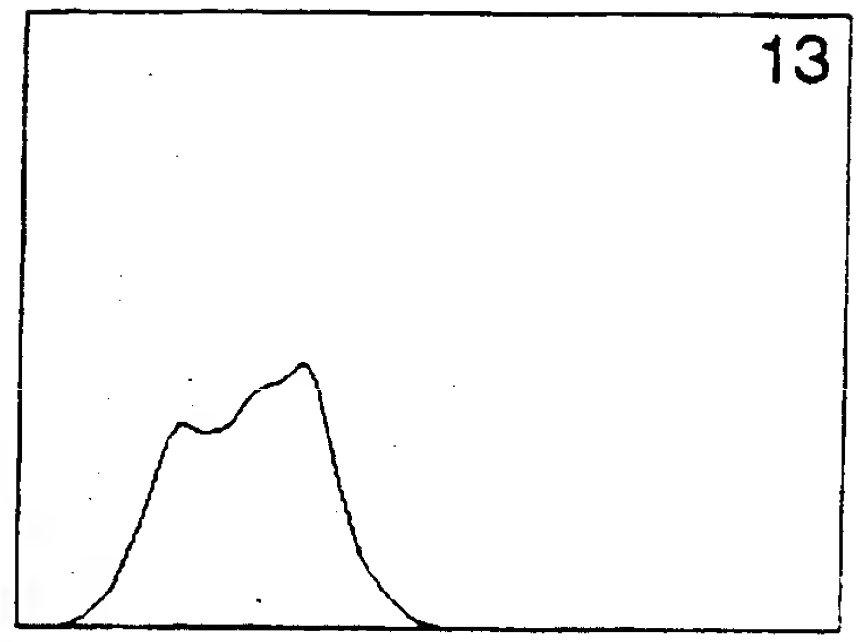


FIGURE 2C

SK.1

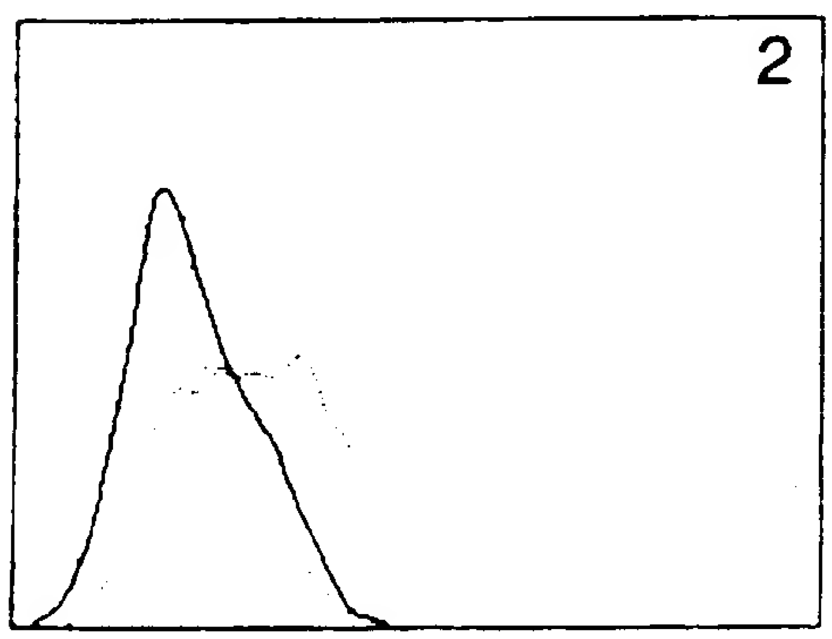


FIGURE 2D

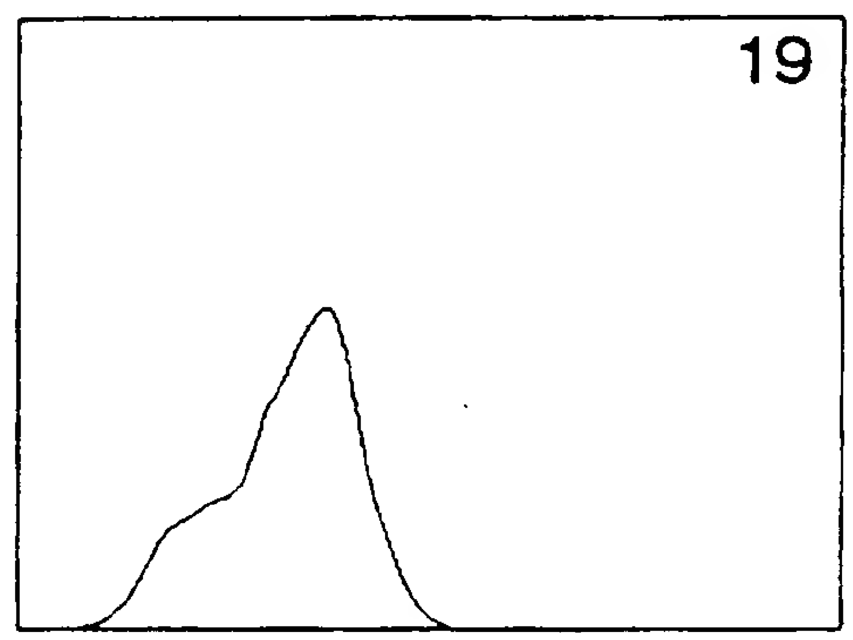


FIGURE 2E

SK.2

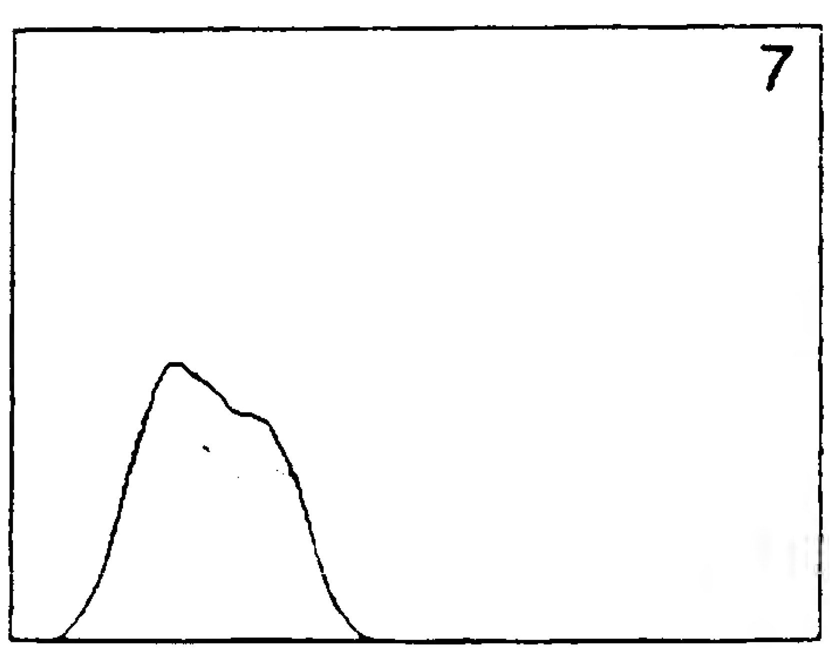


FIGURE 2F

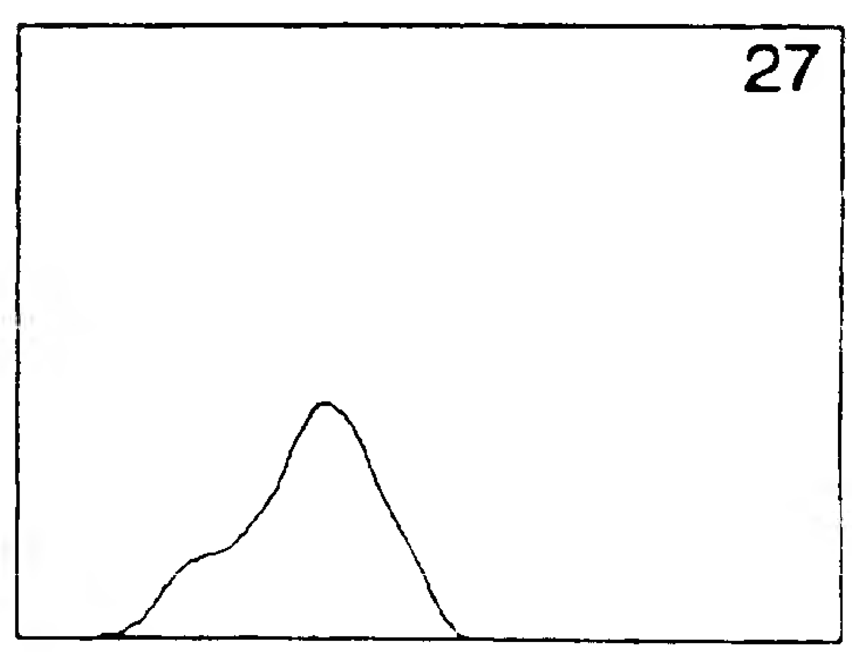
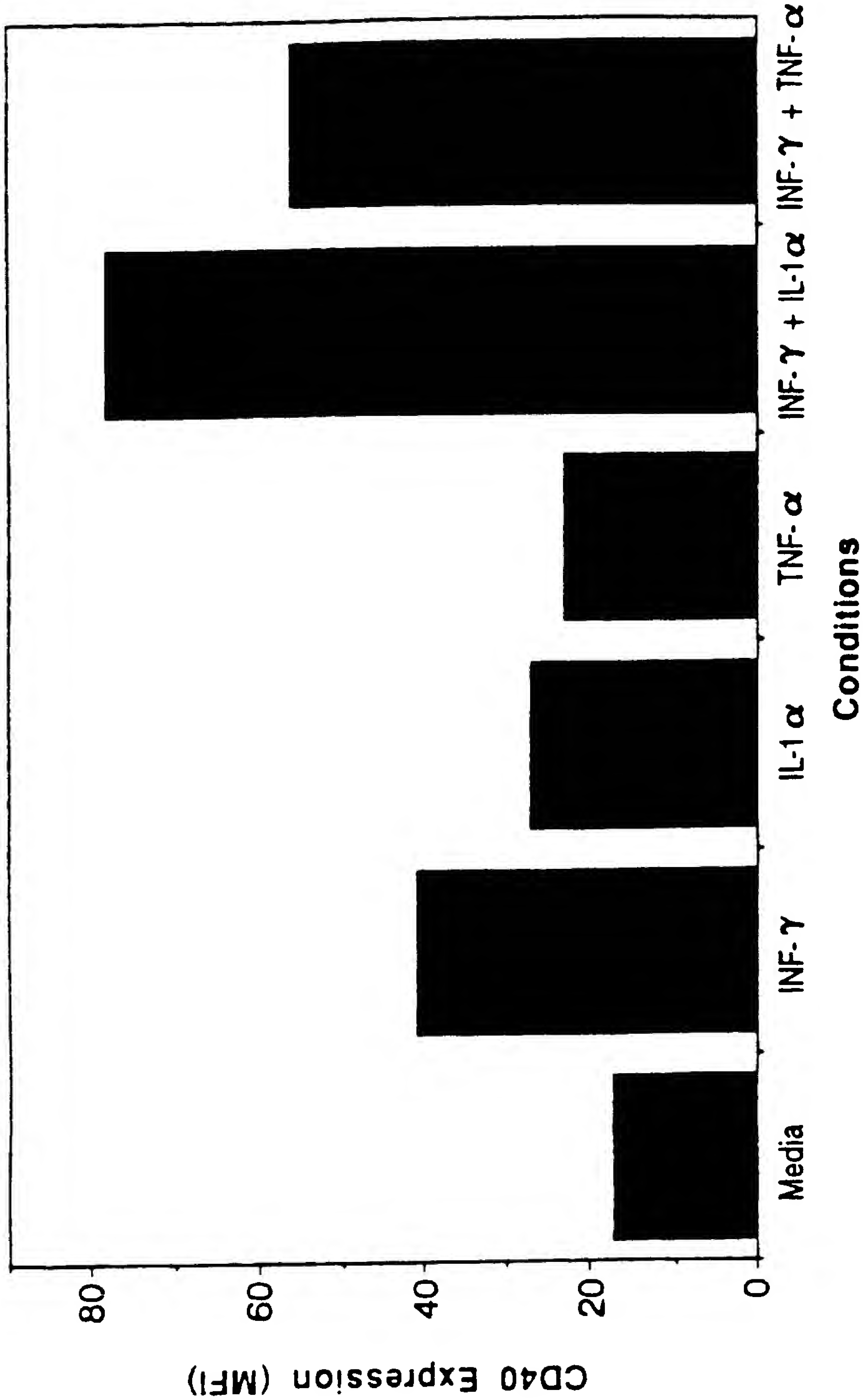


FIGURE 3


CD40 Expression (MFI)
0
20
40
60
80
Media
INF-γ
IL-1α
TNF-α
INF-γ + IL-1α
INF-γ + TNF-α
Conditions

IA.1 cells plus:

Media

D1.1

D1.1
+
Anti-CD40L mAb

D1.1
+
Isotype Control mAb

B2.7

INF-γ

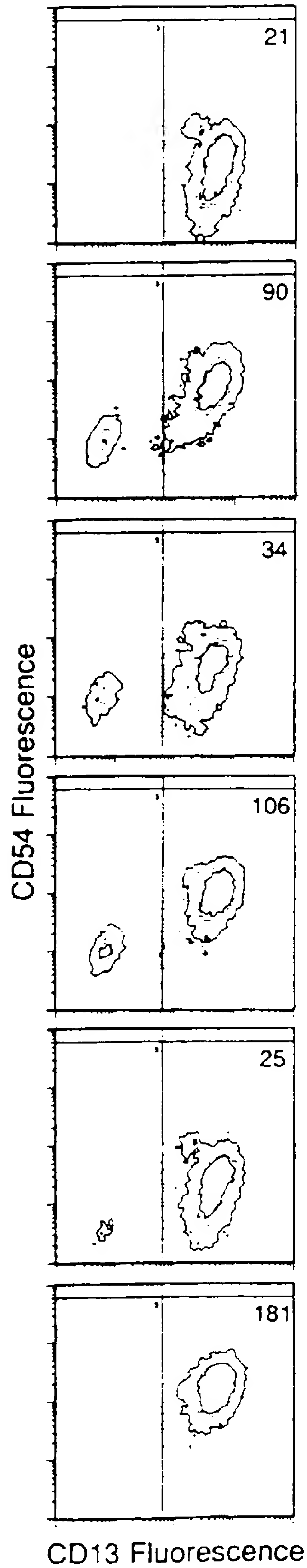


FIGURE 4A

FIGURE 4B

FIGURE 4C

FIGURE 4D

FIGURE 4E

FIGURE 4F

FIGURE 5

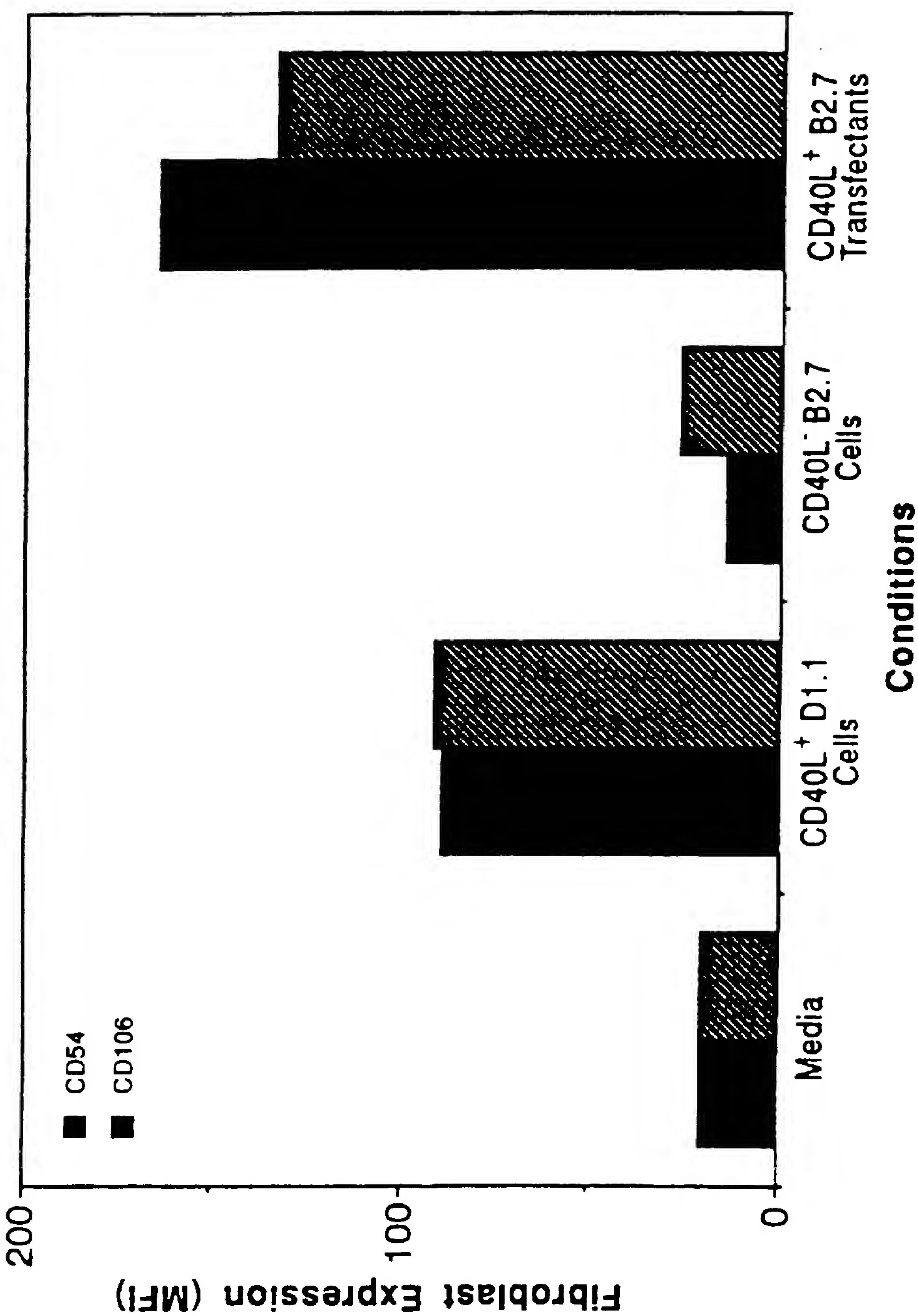
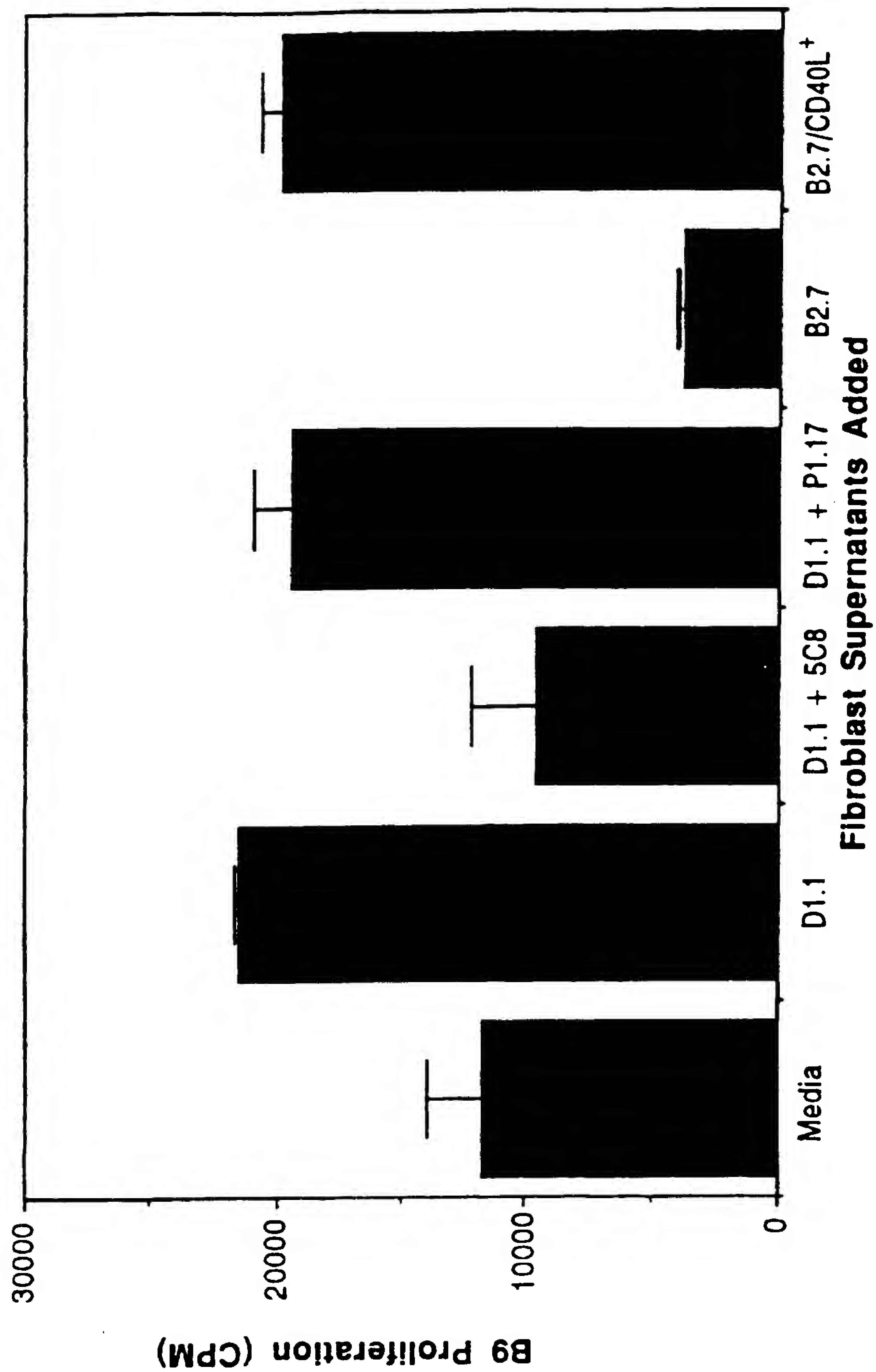

CD54
CD106
Fibroblast Expression (MFI)
200
100
0
Media
CD40L+ D1.1 Cells
CD40L- B2.7 Cells
CD40L+ B2.7 Transfectants
Conditions

FIGURE 6A



B9 Proliferation (CPM)
30000
20000
10000
0
Media
D1.1
D1.1 + 5C8
D1.1 + P1.17
B2.7
B2.7/CD40L+
Fibroblast Supernatants Added

FIGURE 6B

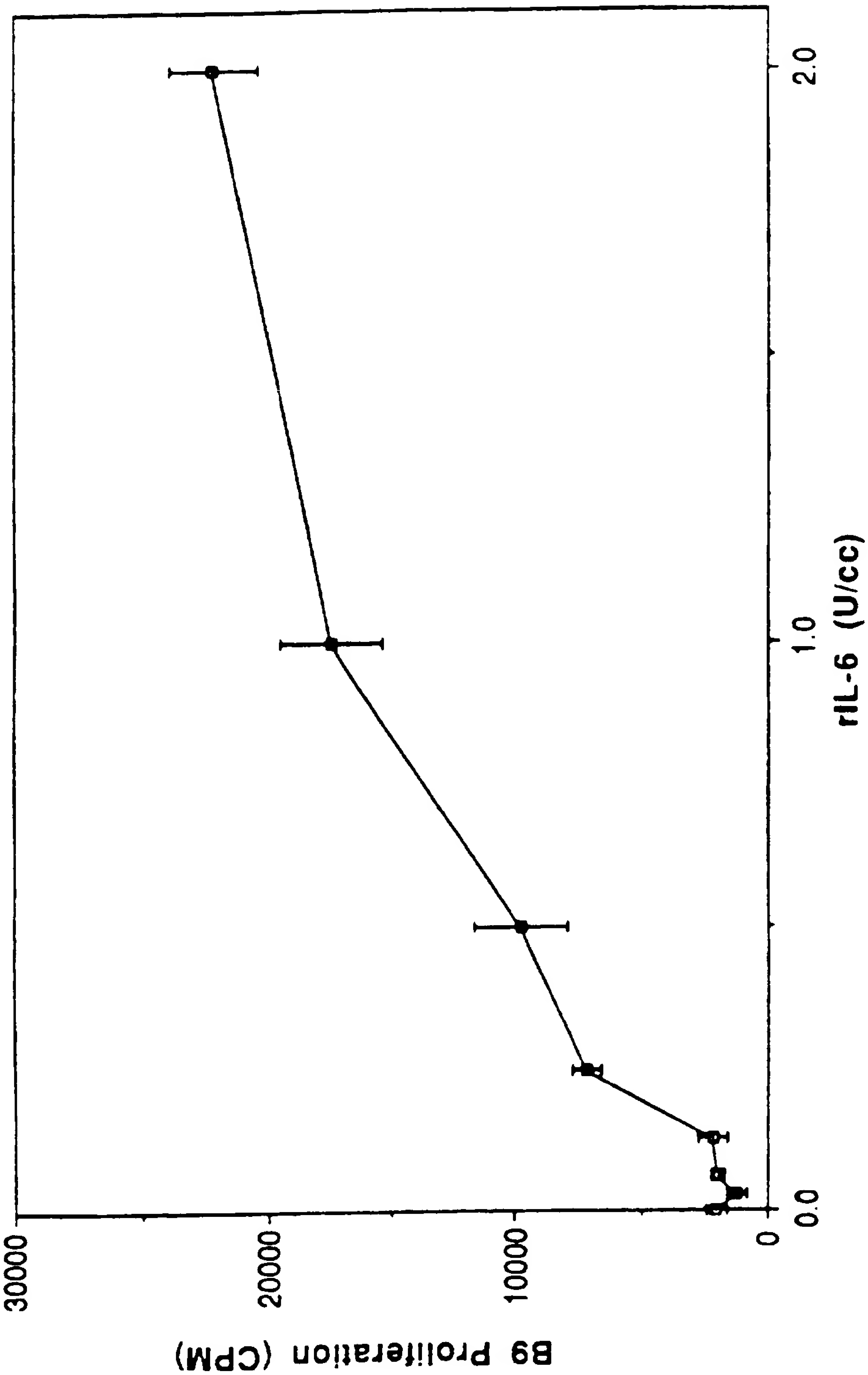


FIGURE 7

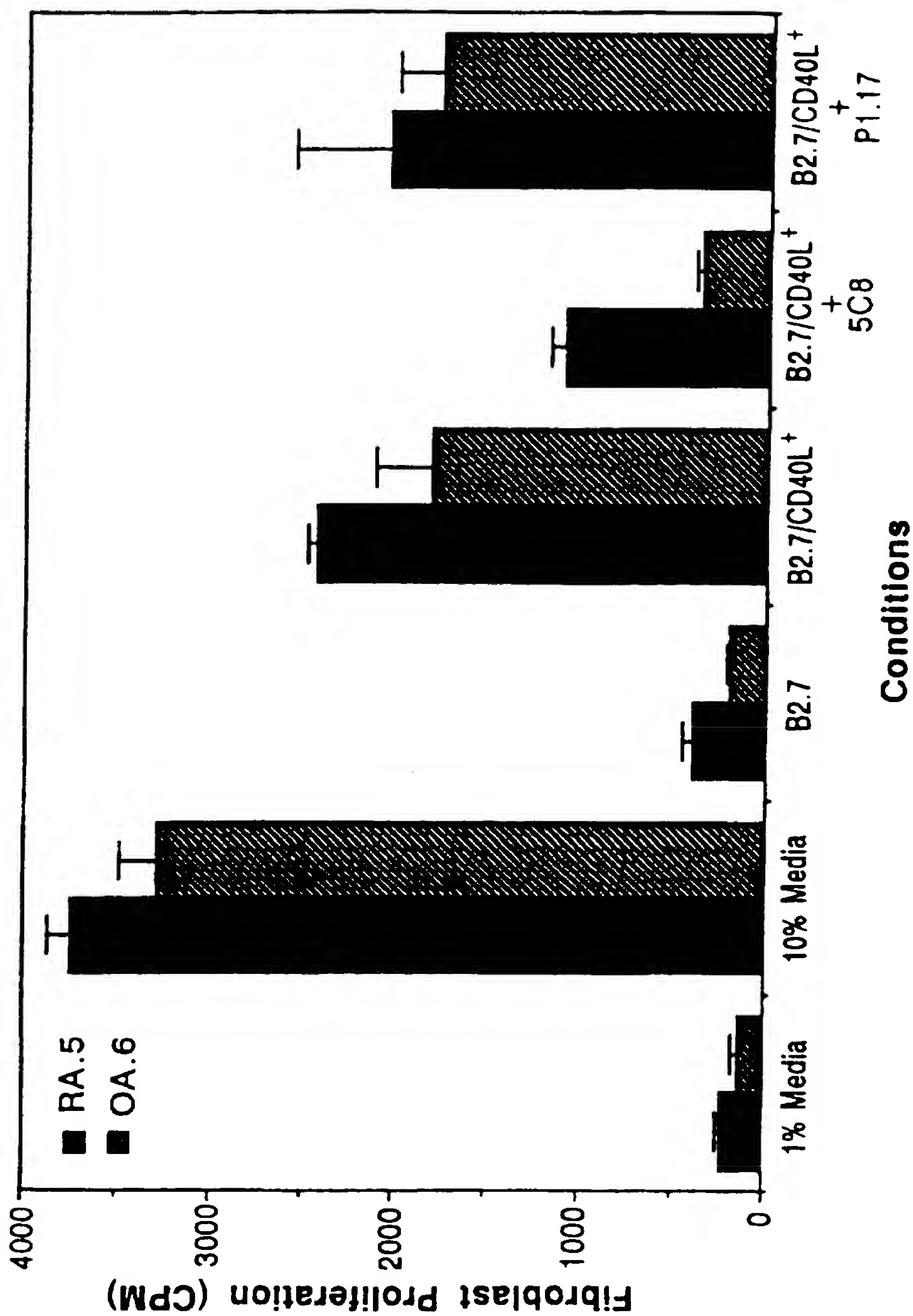
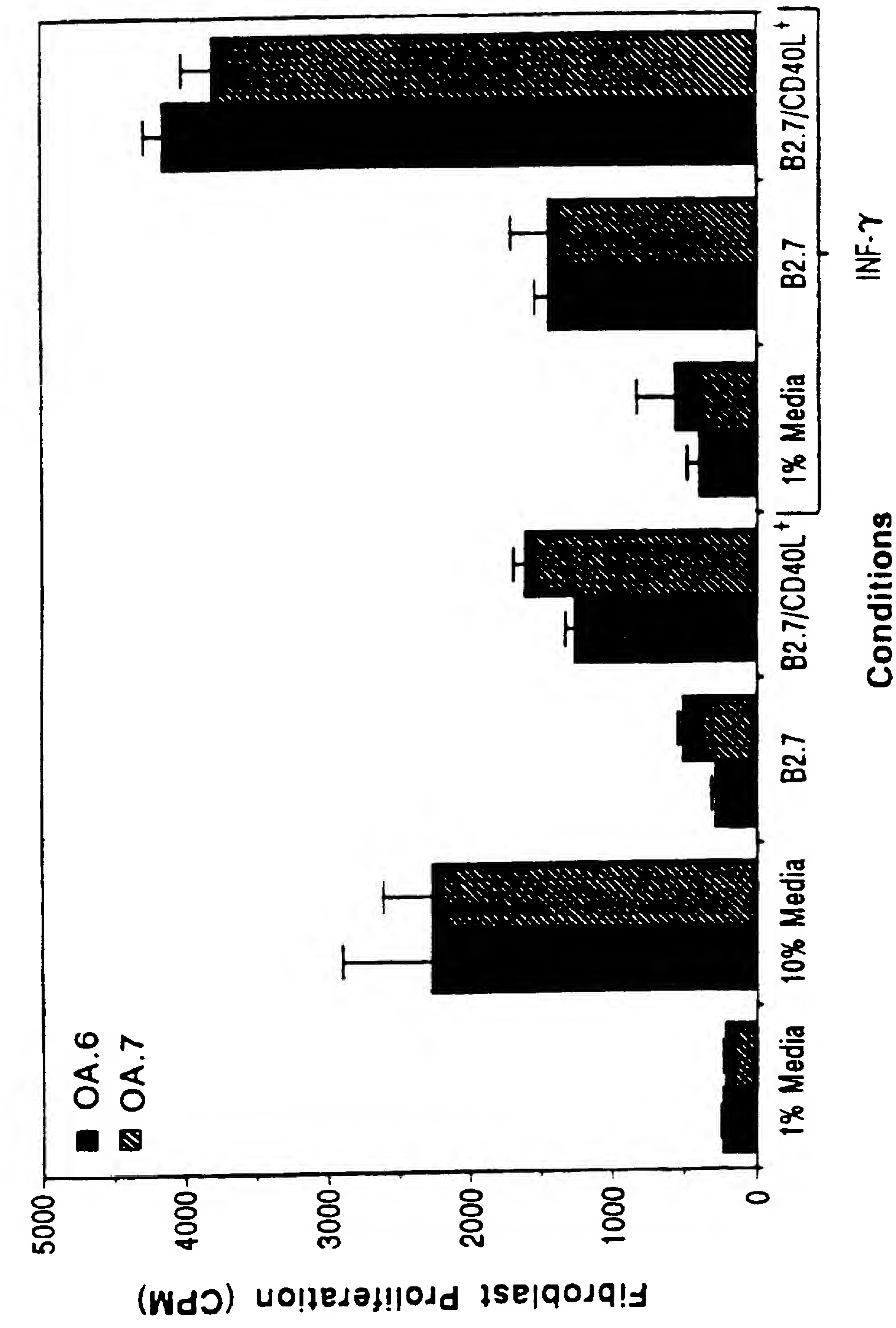

Fibroblast Proliferation (CPM)
4000
3000
2000
1000
0
RA.5
OA.6
1% Media
10% Media
B2.7
B2.7/CD40L+
B2.7/CD40L+ + 5C8
B2.7/CD40L+ + P1.17
Conditions

FIGURE 8



Fibroblast Proliferation (CPM)
5000
4000
3000
2000
1000
0
OA.6
OA.7
1% Media
10% Media
B2.7
B2.7/CD40L+
1% Media
B2.7
B2.7/CD40L+
INF-γ
Conditions

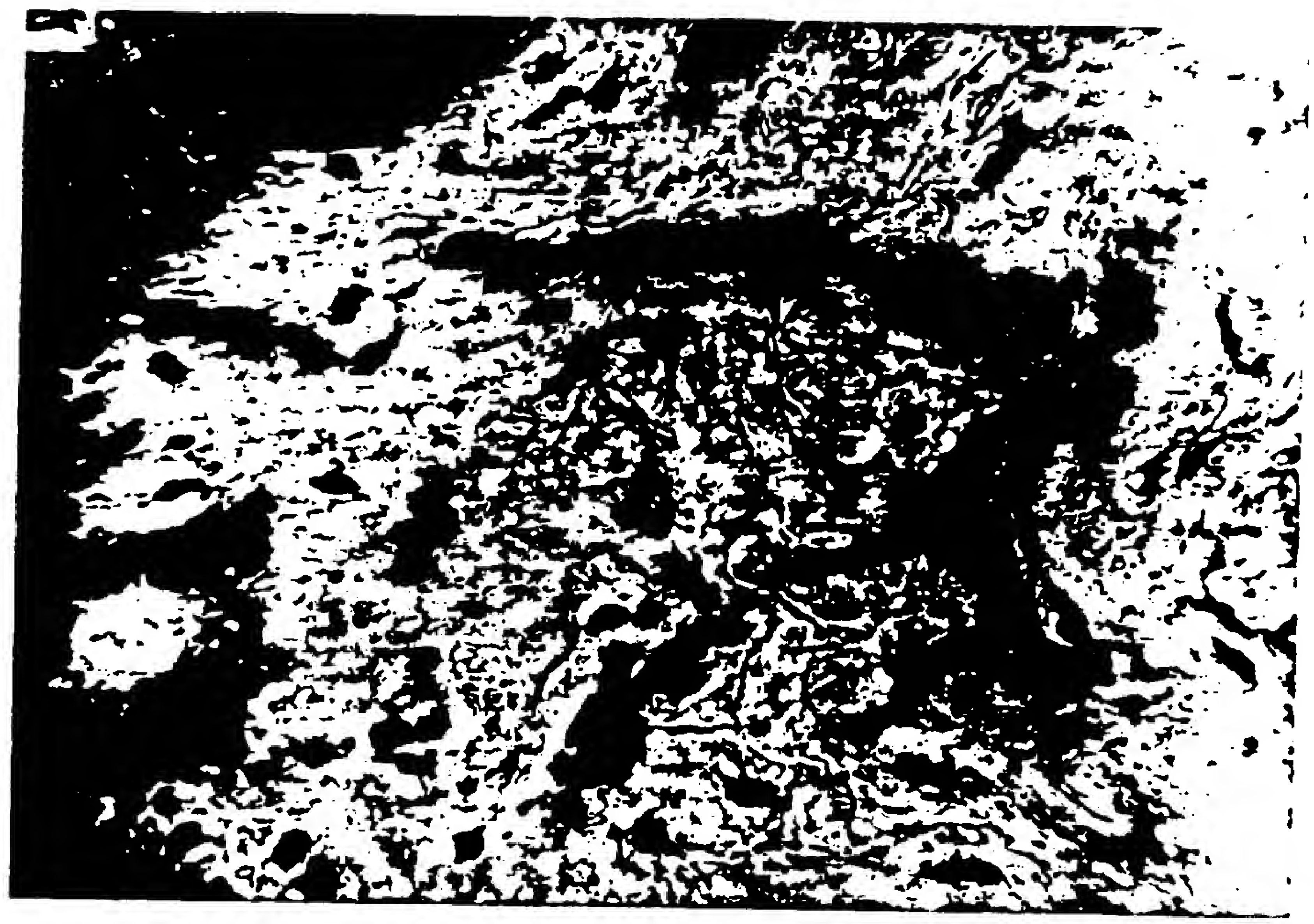


FIGURE 9B

FIGURE 9A

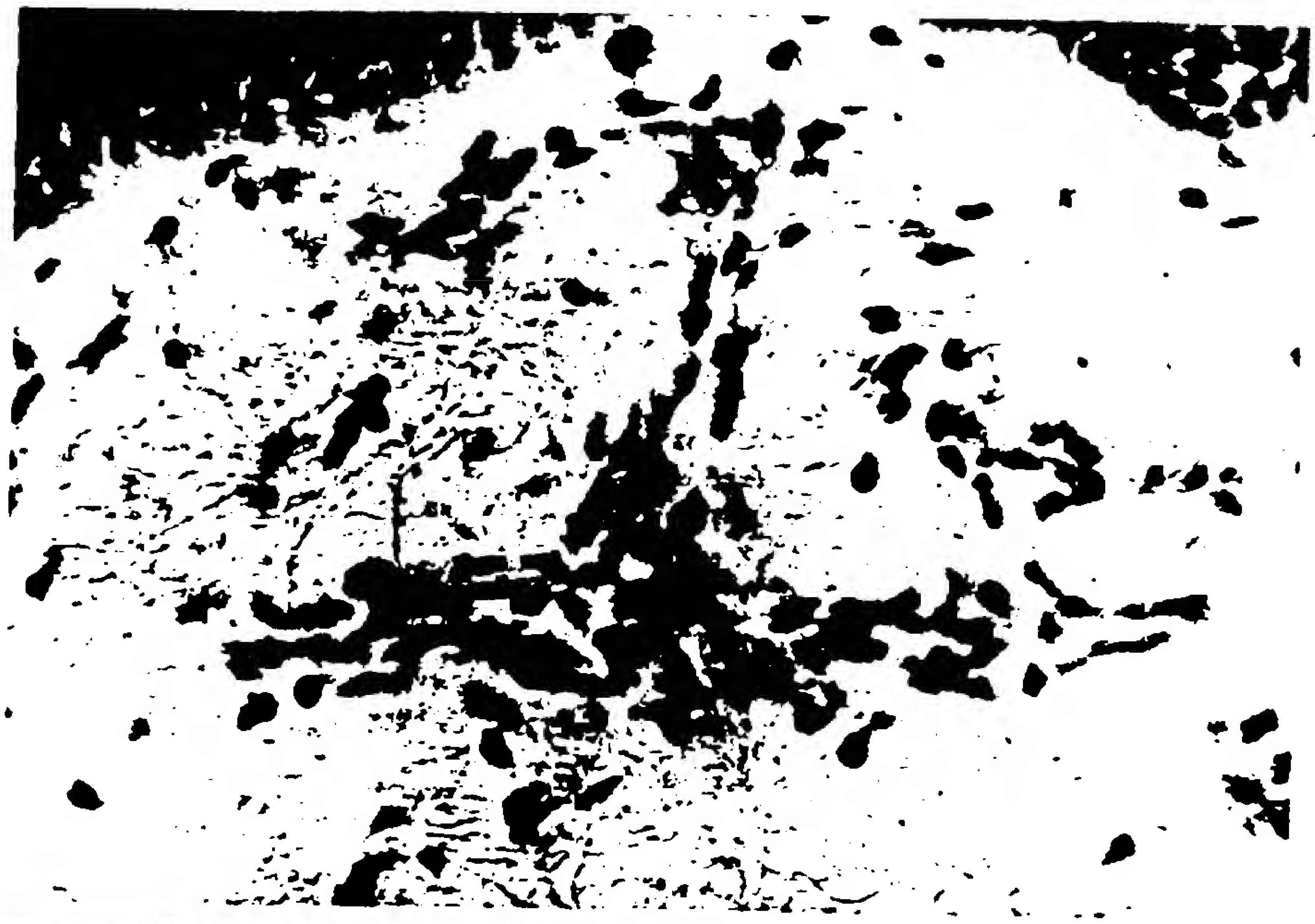


FIGURE 9D

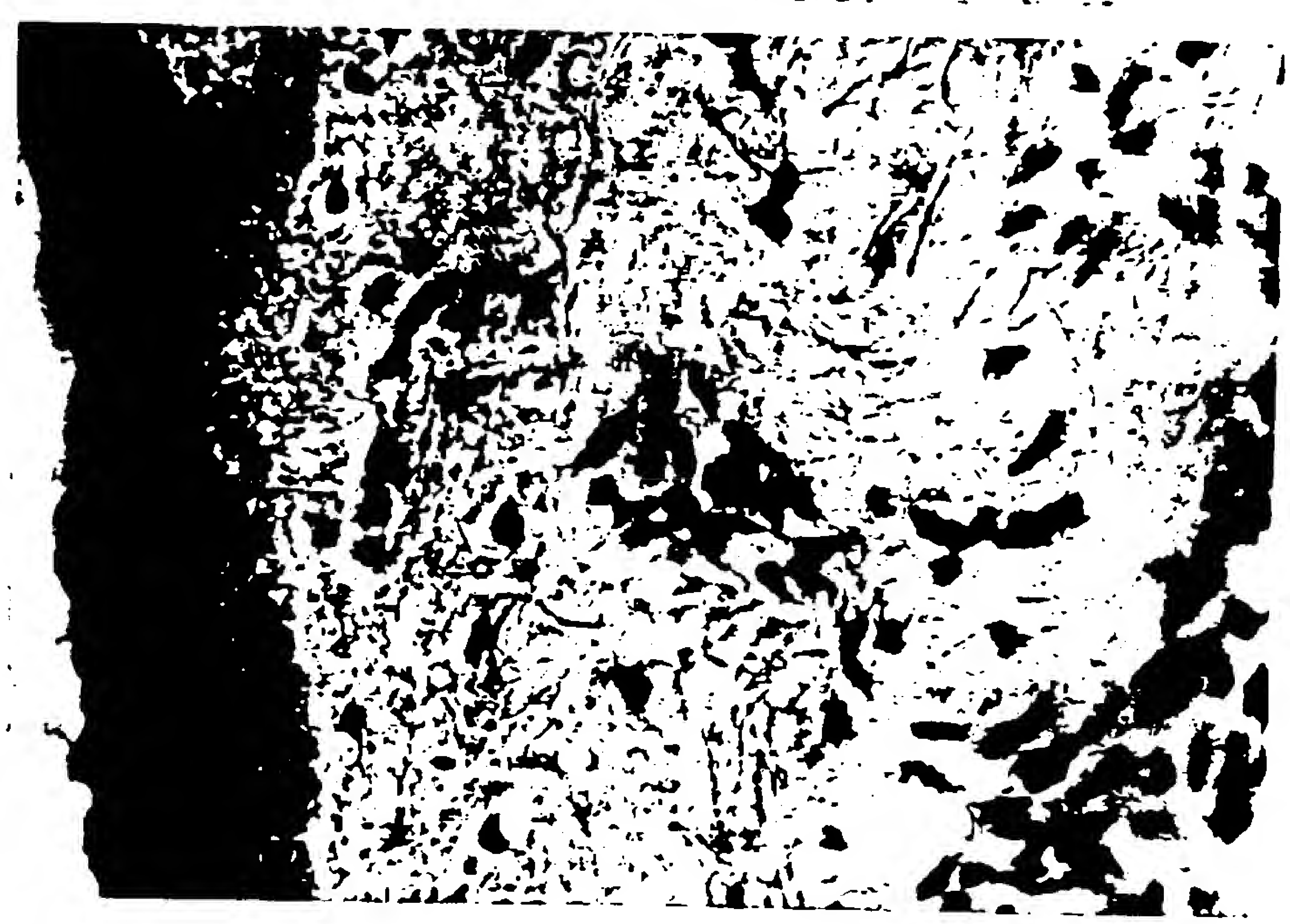


FIGURE 9C

FIGURE 10B

FIGURE 10A

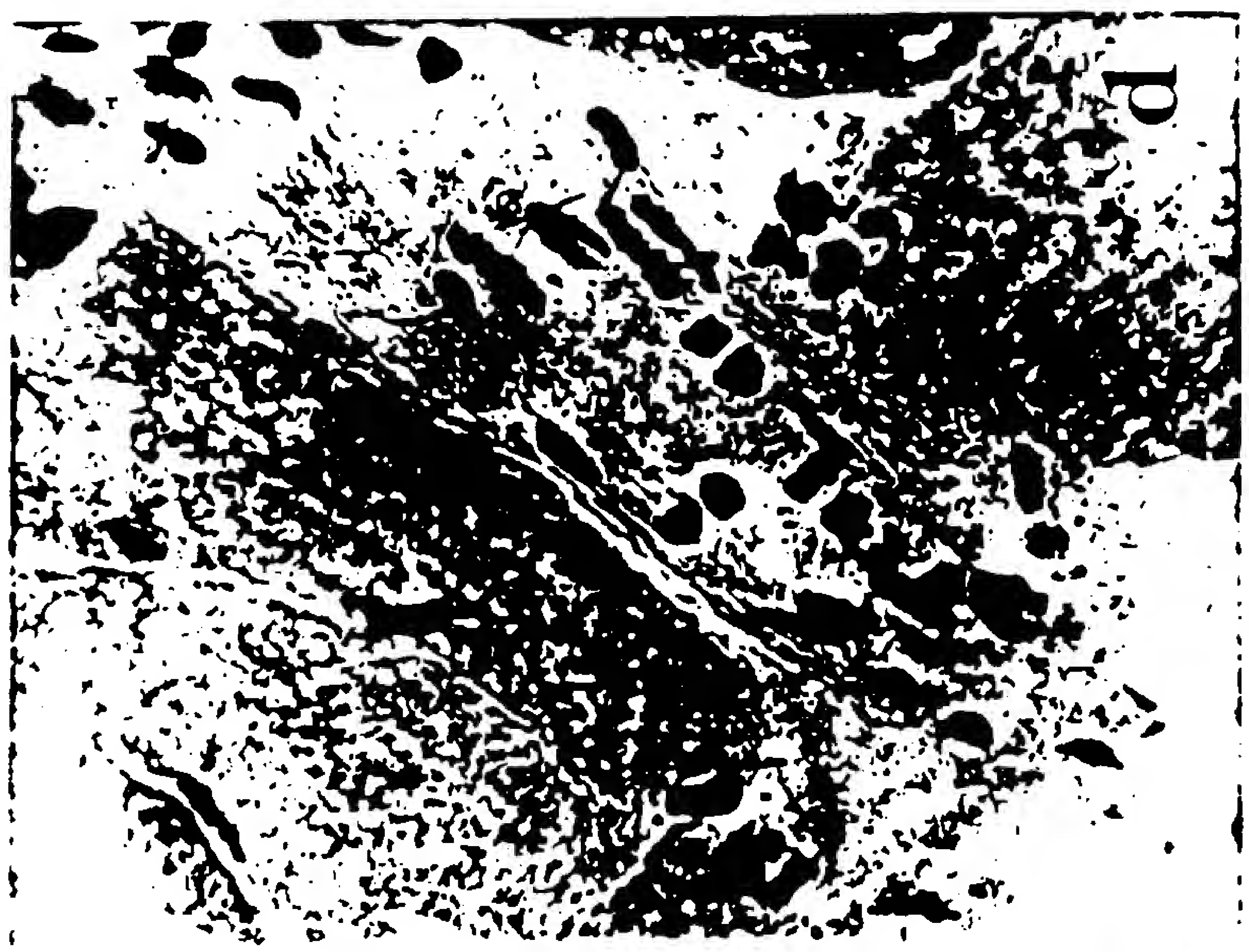


FIGURE 10D

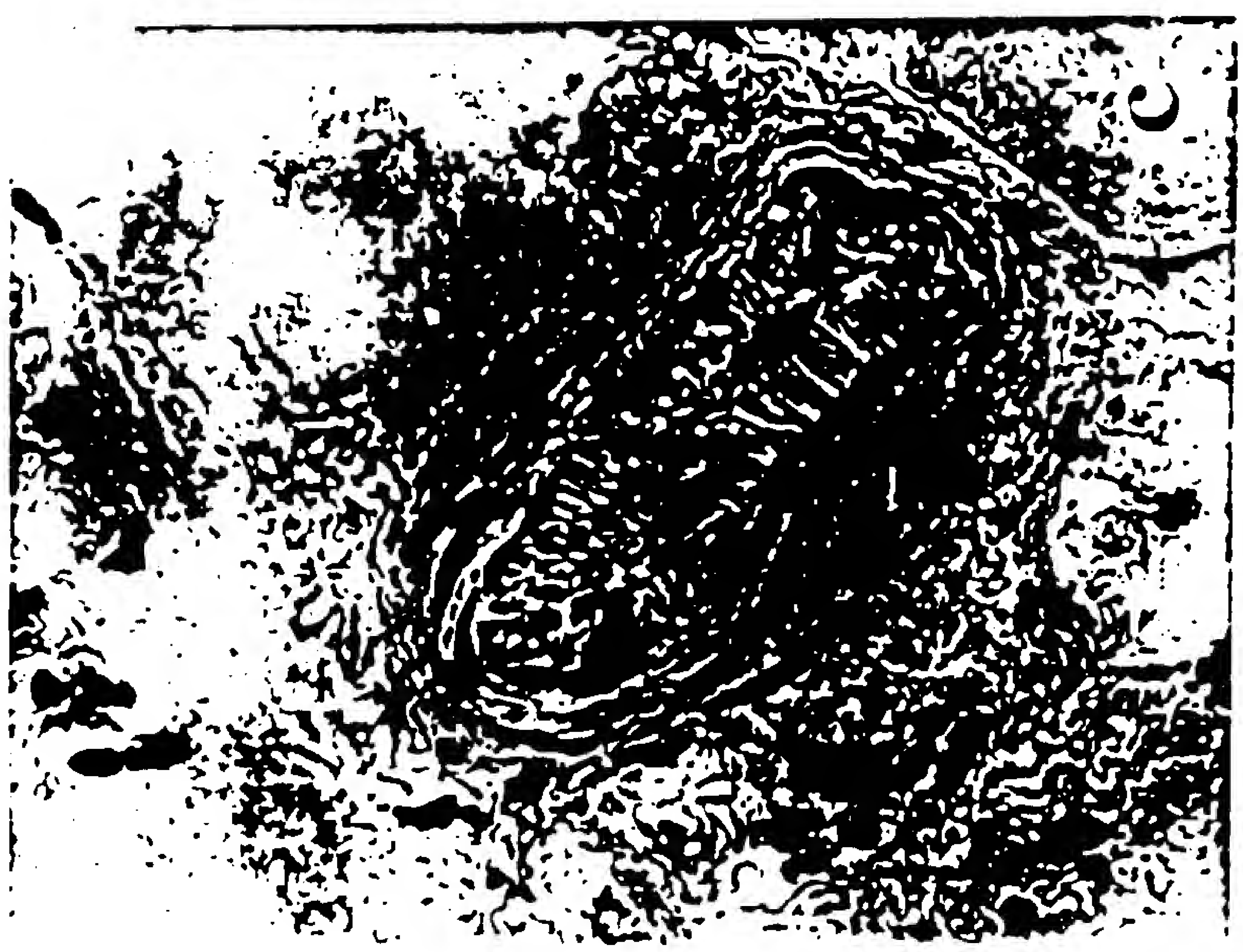


FIGURE 10C

FIGURE 11A

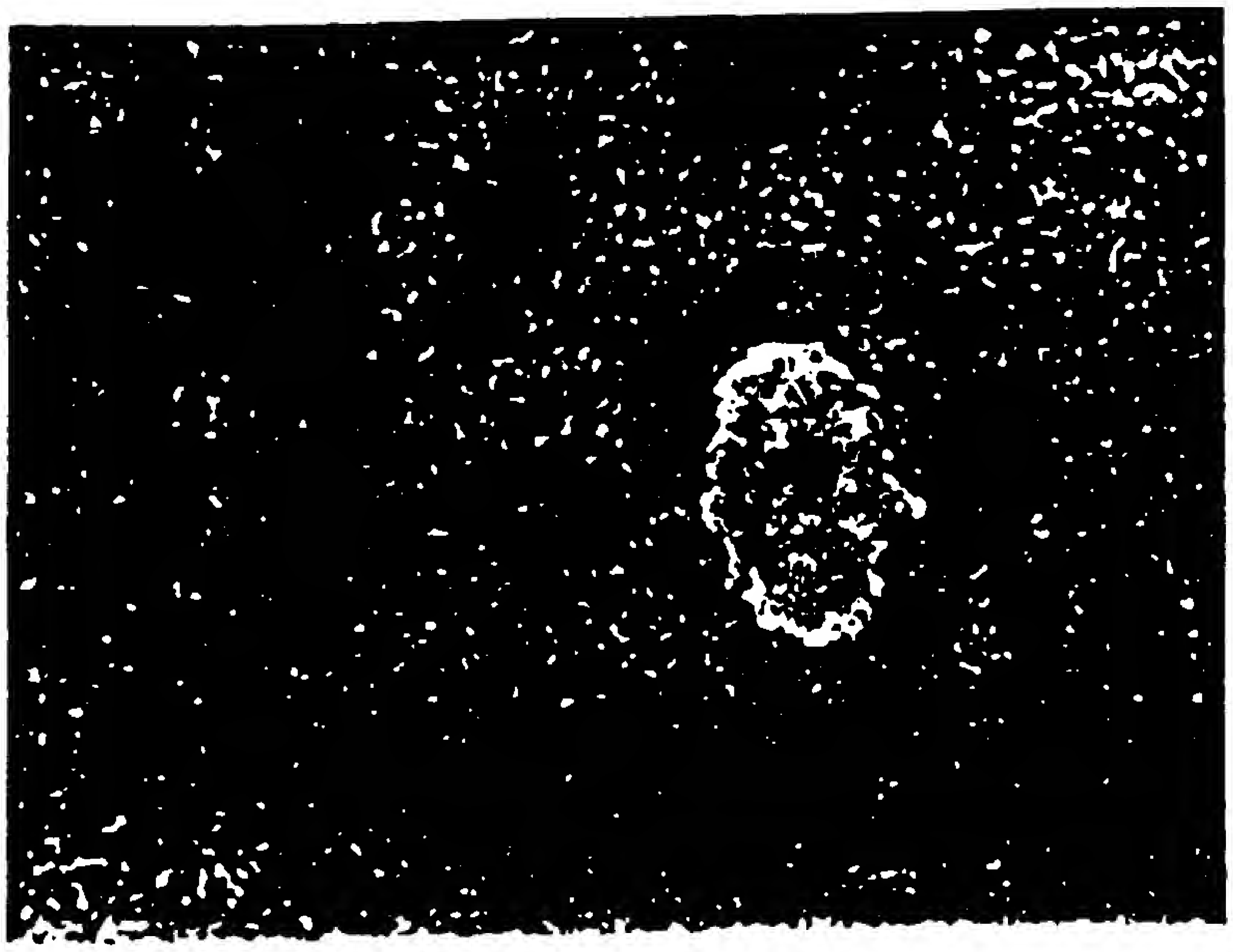
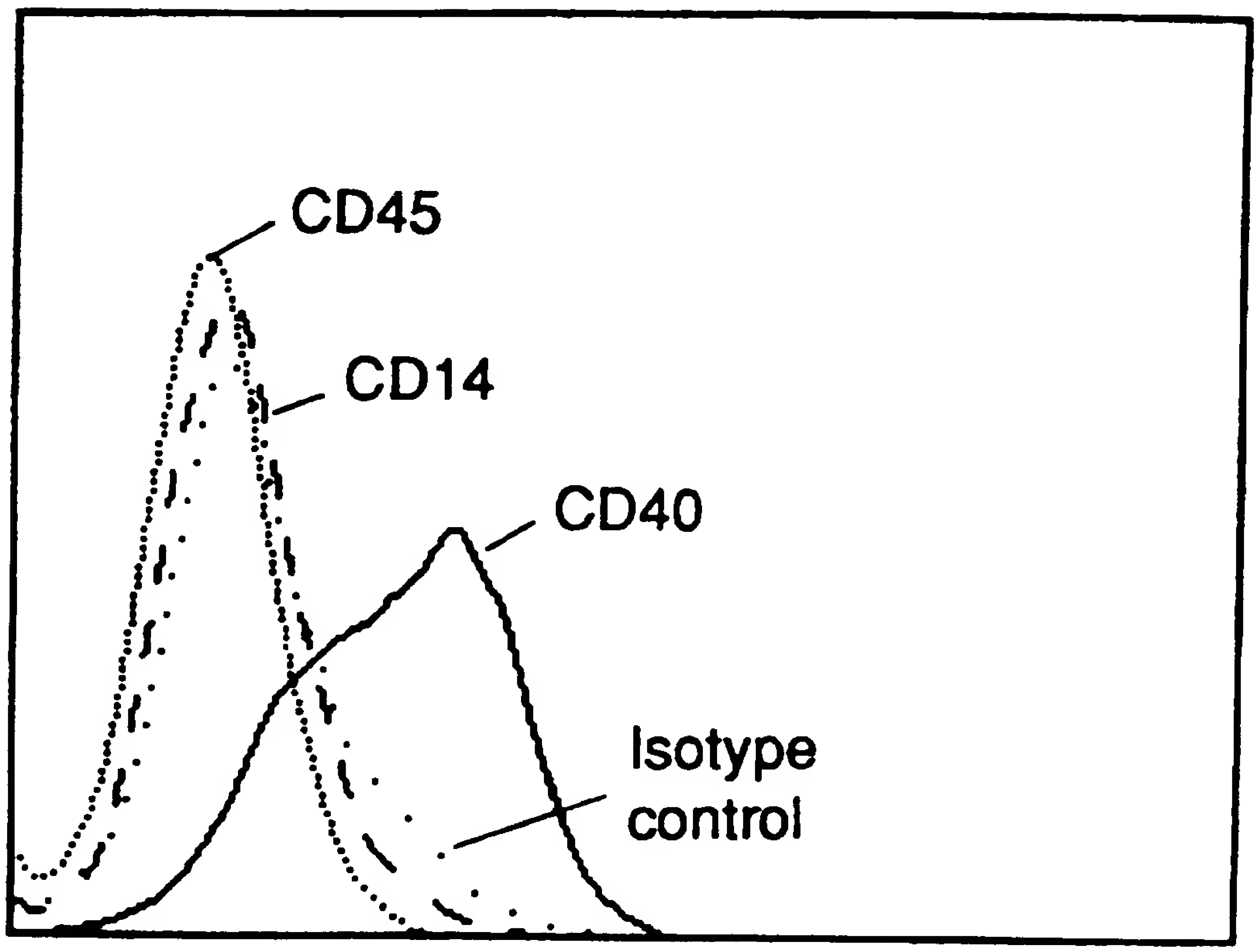


FIGURE 11B

FIGURE 12



Stimulus	mAb
Media	–
rIL-1	–
CD40L^{+} Jurkat D1.1	–
CD40L^{+} Jurkat D1.1	Anti-CD40L
CD40L^{+} Jurkat D1.1	Isotype control
CD40L^{-} Jurkat B2.7	–

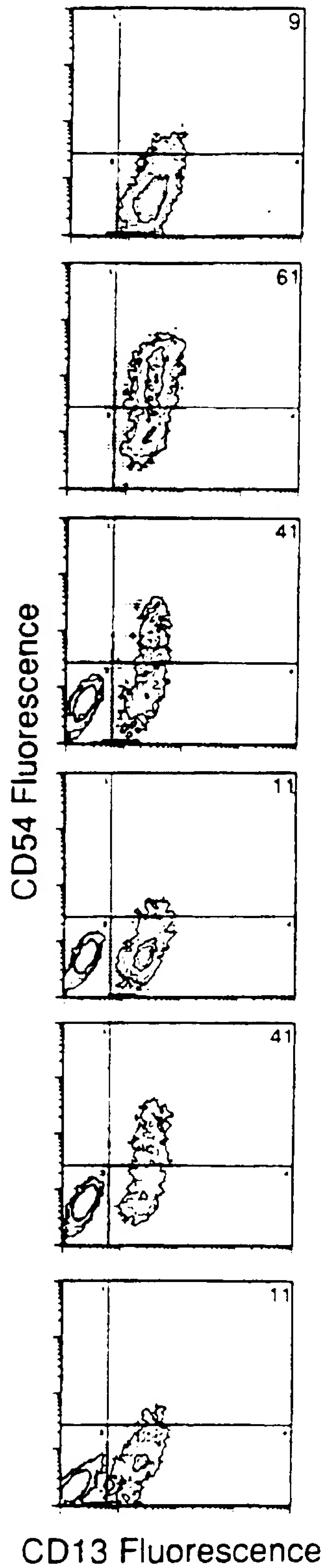


FIGURE 13A

FIGURE 13B

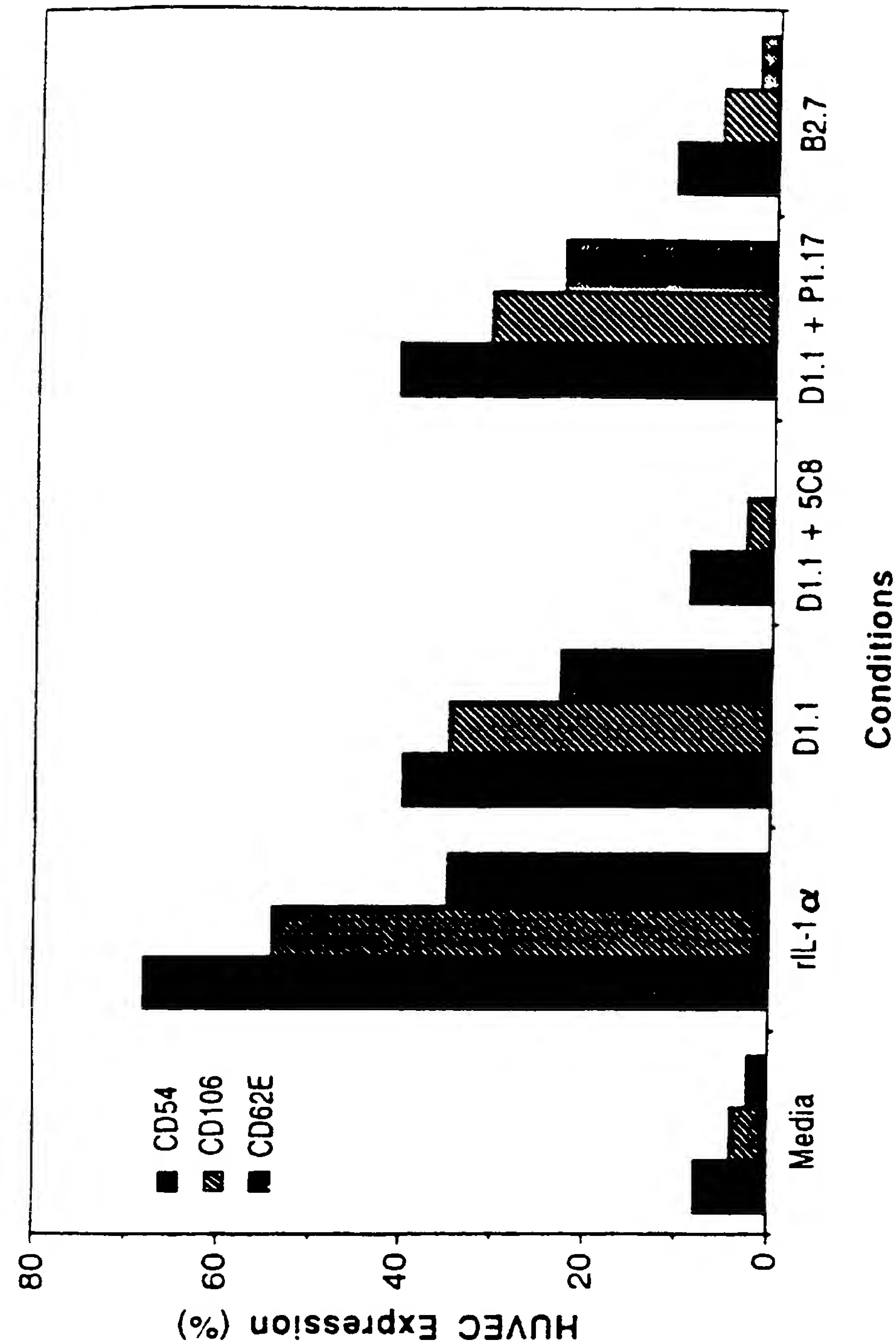
FIGURE 13C

FIGURE 13D

FIGURE 13E

FIGURE 13F

FIGURE 14


CD54
CD106
CD62E
HUVEC Expression (%)
80
60
40
20
0
Media
rIL-1α
D1.1
D1.1 + 5C8
D1.1 + P1.17
B2.7
Conditions

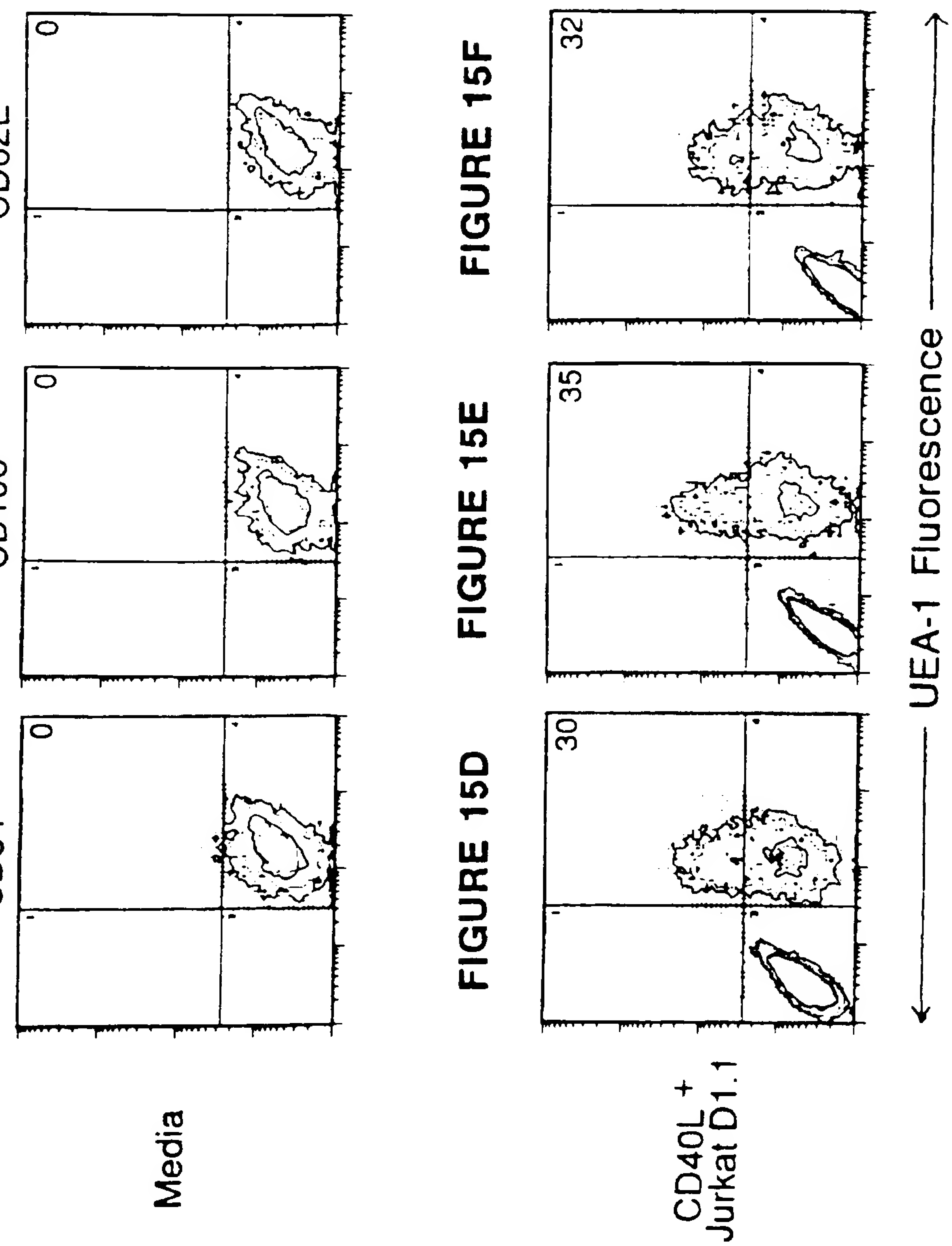


FIGURE 15A FIGURE 15B FIGURE 15C FIGURE 15D FIGURE 15E FIGURE 15F

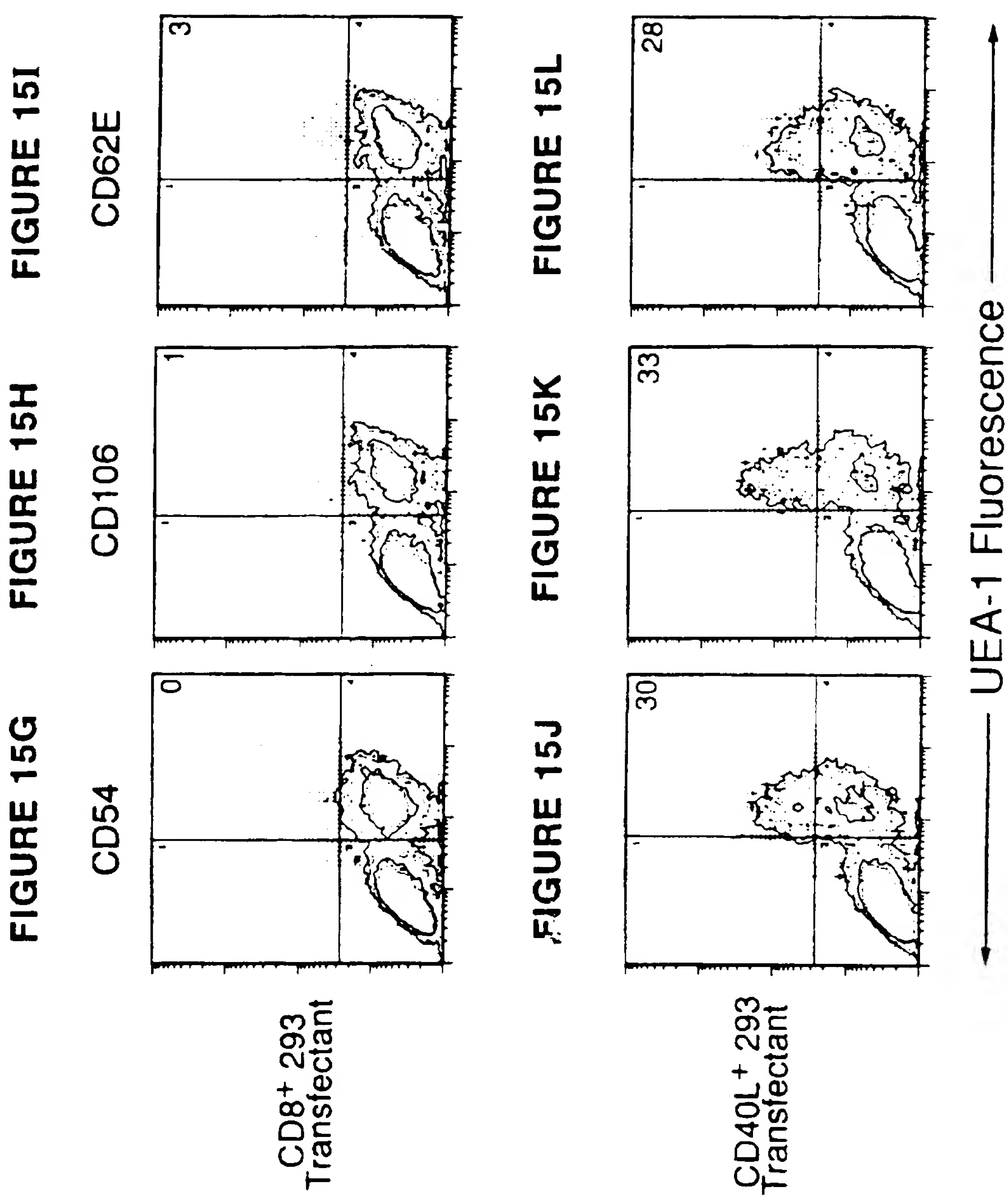


FIGURE 15G FIGURE 15H FIGURE 15I FIGURE 15J FIGURE 15K FIGURE 15L

FIGURE 16A

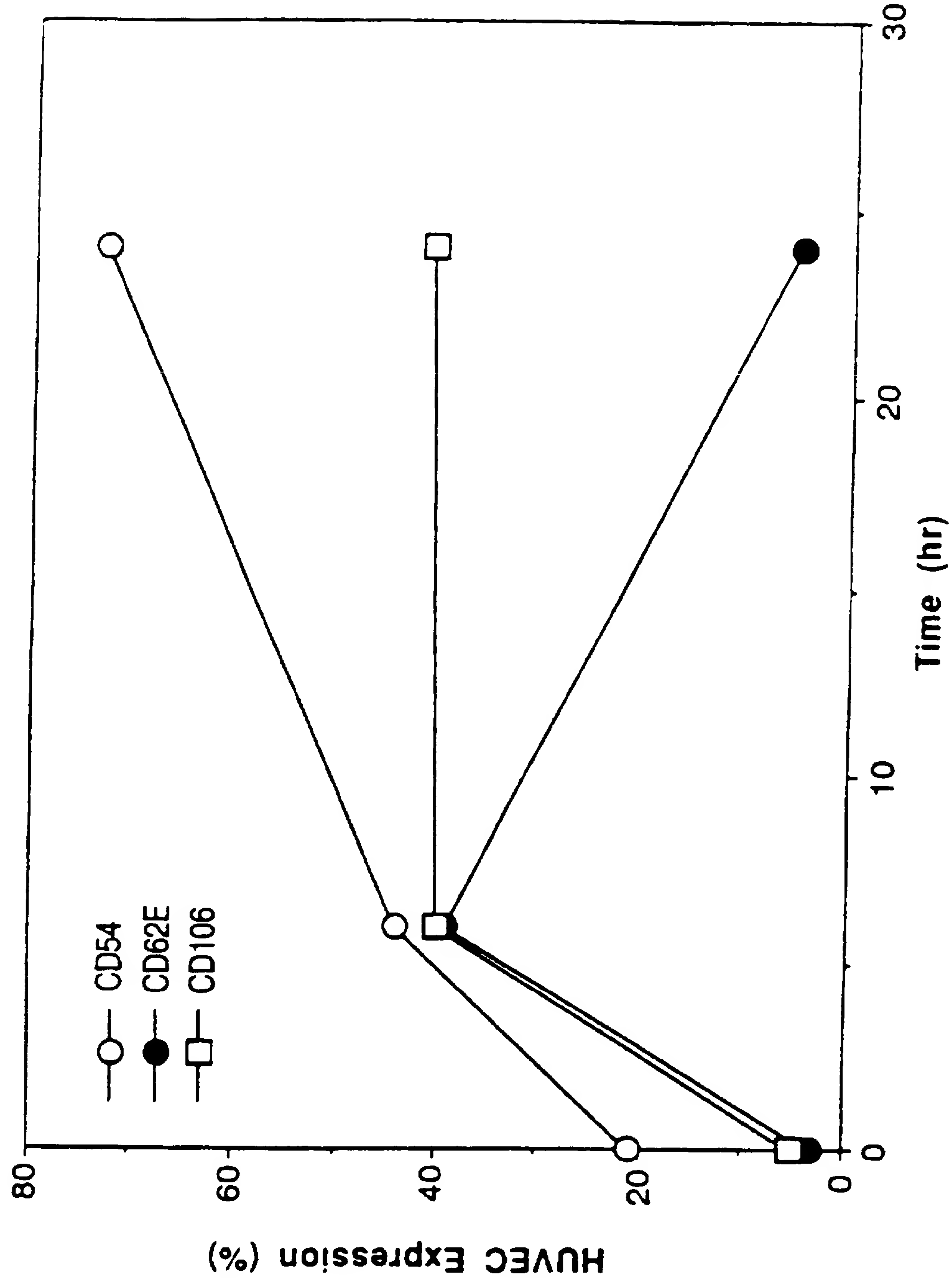

CD54
CD62E
CD106
HUVEC Expression (%)
Time (hr)
0
20
40
60
80
0
10
20
30

FIGURE 16B

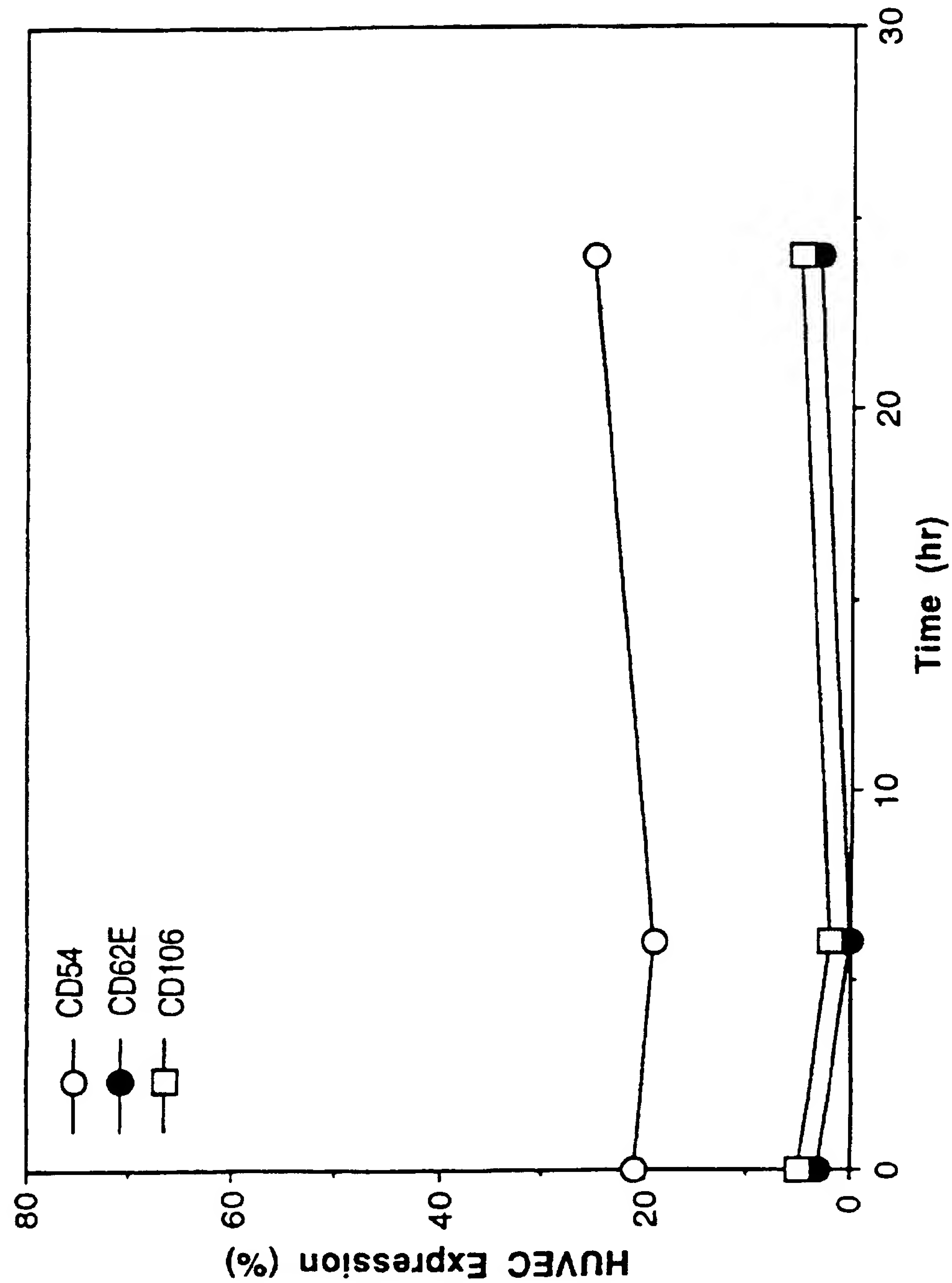

CD54
CD62E
CD106
HUVEC Expression (%)
80
60
40
20
0
Time (hr)
0
10
20
30

FIGURE 17A

REMARKS ATOMIC COORDINATES OF CD40L CRYSTAL STRUCTURE IN PDB FORMAT

CRYST 77.170 77.170 90.460 90.00 90.00 120.00 R3

ATOM	1	N	GLY	116	-7.954	-16.144	22.488	1.00	64.71	A
ATOM	2	HT1	GLY	116	-7.087	-15.852	21.964	1.00	15.00	A
ATOM	3	HT2	GLY	116	-8.082	-17.142	22.242	1.00	15.00	A
ATOM	4	HT3	GLY	116	-8.630	-15.576	21.928	1.00	15.00	A
ATOM	5	CA	GLY	116	-7.927	-15.755	23.928	1.00	64.37	A
ATOM	6	C	GLY	116	-6.990	-16.621	24.780	1.00	64.34	A
ATOM	7	O	GLY	116	-6.968	-17.814	24.563	1.00	64.44	A
ATOM	8	N	ASP	117	-6.238	-16.043	25.740	1.00	64.04	A
ATOM	9	H	ASP	117	-5.617	-16.709	26.170	1.00	15.00	A
ATOM	10	CA	ASP	117	-6.284	-14.616	26.130	1.00	63.57	A
ATOM	11	CB	ASP	117	-5.711	-14.402	27.539	1.00	63.36	A
ATOM	12	CG	ASP	117	-6.518	-15.163	28.574	1.00	63.71	A
ATOM	13	OD1	ASP	117	-6.090	-16.247	28.965	1.00	63.24	A
ATOM	14	OD2	ASP	117	-7.566	-14.668	28.987	1.00	63.29	A
ATOM	15	C	ASP	117	-5.651	-13.585	25.184	1.00	63.31	A
ATOM	16	O	ASP	117	-6.039	-12.427	25.145	1.00	63.35	A
ATOM	17	N	GLN	118	-4.713	-14.090	24.379	1.00	62.72	A
ATOM	18	H	GLN	118	-4.450	-15.040	24.541	1.00	15.00	A
ATOM	19	CA	GLN	118	-4.097	-13.313	23.281	1.00	61.79	A
ATOM	20	CB	GLN	118	-2.918	-14.117	22.687	1.00	62.46	A
ATOM	21	CG	GLN	118	-3.047	-15.659	22.562	1.00	62.95	A
ATOM	22	CD	GLN	118	-4.277	-16.118	21.790	1.00	63.26	A
ATOM	23	OE1	GLN	118	-5.396	-16.000	22.277	1.00	63.43	A
ATOM	24	NE2	GLN	118	-4.044	-16.665	20.601	1.00	63.42	A
ATOM	25	HE21	GLN	118	-4.836	-16.715	19.975	1.00	15.00	A
ATOM	26	HE22	GLN	118	-3.151	-16.995	20.298	1.00	15.00	A
ATOM	27	C	GLN	118	-4.999	-12.841	22.128	1.00	60.59	A
ATOM	28	O	GLN	118	-4.887	-13.379	21.052	1.00	60.79	A
ATOM	29	N	ASN	119	-5.912	-11.901	22.445	1.00	58.61	A
ATOM	30	H	ASN	119	-5.917	-11.600	23.389	1.00	15.00	A
ATOM	31	CA	ASN	119	-6.689	-11.222	21.386	1.00	56.39	A
ATOM	32	CB	ASN	119	-7.947	-11.982	20.936	1.00	56.95	A
ATOM	33	CG	ASN	119	-7.652	-13.352	20.375	1.00	57.45	A
ATOM	34	OD1	ASN	119	-7.941	-14.303	21.084	1.00	58.50	A
ATOM	35	ND2	ASN	119	-7.005	-13.431	19.241	1.00	58.58	A
ATOM	36	HD21	ASN	119	-6.843	-12.617	18.646	1.00	15.00	A
ATOM	37	HD22	ASN	119	-6.740	-14.221	18.684	1.00	15.00	A
ATOM	38	C	ASN	119	-7.053	-9.724	21.571	1.00	53.62	A
ATOM	39	O	ASN	119	-6.746	-8.933	20.694	1.00	56.55	A
ATOM	40	N	PRO	120	-7.737	-9.288	22.698	1.00	50.17	A
ATOM	41	CD	PRO	120	-8.151	-10.129	23.810	1.00	51.90	A
ATOM	42	CA	PRO	120	-8.402	-7.945	22.818	1.00	48.19	A
ATOM	43	CB	PRO	120	-9.191	-8.008	24.117	1.00	47.42	A
ATOM	44	CG	PRO	120	-9.444	-9.493	24.321	1.00	51.93	A
ATOM	45	C	PRO	120	-7.750	-6.524	22.657	1.00	45.59	A
ATOM	46	O	PRO	120	-8.187	-5.516	23.225	1.00	45.37	A
ATOM	47	N	GLN	121	-6.789	-6.458	21.721	1.00	38.52	A
ATOM	48	H	GLN	121	-6.287	-7.704	21.5[illegible]	1.00	15.00	A
ATOM	49	CA	GLN	121	-6.733	-5.359	20.753	1.00	29.14	A
ATOM	50	CB	GLN	121	-5.454	-5.735	19.971	1.00	26.30	A
ATOM	51	CG	GLN	121	-5.128	-4.943	18.710	1.00	26.84	A
ATOM	52	CD	GLN	121	-4.923	-3.460	18.949	1.00	27.26	A
ATOM	53	OE1	GLN	121	-5.822	-2.668	18.709	1.00	28.66	A
ATOM	54	NE2	GLN	121	-3.717	-3.100	19.341	1.00	33.90	A
ATOM	55	HE21	GLN	121	2.883	-3.614	19.564	1.00	15.00	A
ATOM	56	HE22	GLN	121	-3.442	-2.138	19.204	1.00	15.00	A
ATOM	57	C	GLN	121	-8.065	-5.218	19.903	1.00	26.33	A
ATOM	58	O	GLN	121	-8.905	-6.097	19.834	1.00	21.41	A
ATOM	59	N	ILE	122	-8.288	-4.051	19.272	1.00	21.21	A

FIGURE 17B

ATOM	60	H	ILE	122	-7.600	-3.320	19.337	1.00	15.00	A
ATOM	61	CA	ILE	122	-9.383	-3.952	18.295	1.00	[illegible]	A
ATOM	62	CB	ILE	122	-10.238	-2.629	18.396	1.00	[illegible]	A
ATOM	63	CG2	ILE	122	-11.275	-2.428	17.272	1.00	21.61	A
ATOM	64	CG1	ILE	122	-11.076	-2.744	19.668	1.00	24.13	A
ATOM	65	CD1	ILE	122	-11.751	-1.440	20.073	1.00	23.04	A
ATOM	66	C	ILE	122	-8.833	-4.108	16.895	1.00	18.96	A
ATOM	67	O	ILE	122	-8.135	-3.243	16.379	1.00	17.93	A
ATOM	68	N	ALA	123	-9.159	-5.240	16.283	1.00	14.72	A
ATOM	69	H	ALA	123	-9.599	-5.978	16.805	1.00	15.00	A
ATOM	70	CA	ALA	123	-8.656	-5.401	14.917	1.00	14.29	A
ATOM	71	CB	ALA	123	-7.176	-5.868	14.903	1.00	12.83	A
ATOM	72	C	ALA	123	-9.483	-6.315	13.985	1.00	15.66	A
ATOM	73	O	ALA	123	-10.170	-7.261	14.323	1.00	13.58	A
ATOM	74	N	ALA	124	-9.388	-6.009	12.724	1.00	13.45	A
ATOM	75	H	ALA	124	-8.894	-5.185	12.456	1.00	15.00	A
ATOM	76	CA	ALA	124	-10.087	-6.920	11.836	1.00	14.55	A
ATOM	77	CB	ALA	124	-11.486	-6.368	11.446	1.00	11.37	A
ATOM	78	C	ALA	124	-9.271	-7.123	10.563	1.00	13.54	A
ATOM	79	O	ALA	124	-8.501	-6.274	10.129	1.00	16.29	A
ATOM	80	N	HIS	125	-9.544	-8.248	9.937	1.00	11.49	A
ATOM	81	H	HIS	125	-10.100	-8.900	10.426	1.00	15.00	A
ATOM	82	CA	HIS	125	-9.100	-8.524	8.590	1.00	11.51	A
ATOM	83	CB	HIS	125	-7.605	-8.908	8.614	1.00	11.43	A
ATOM	84	CG	HIS	125	-7.119	-9.116	7.205	1.00	7.41	A
ATOM	85	ND1	HIS	125	-6.750	-8.130	6.421	1.00	6.60	A
ATOM	86	HD1	HIS	125	-6.708	-7.168	6.621	1.00	15.00	A
ATOM	87	CD2	HIS	125	-7.075	-10.291	6.456	1.00	12.36	A
ATOM	88	NE2	HIS	125	-6.670	-9.971	5.234	1.00	6.20	A
ATOM	89	CE1	HIS	125	-6.462	-8.646	5.211	1.00	4.48	A
ATOM	90	C	HIS	125	-10.024	-9.570	7.931	1.00	12.63	A
ATOM	91	O	HIS	125	-10.324	-10.650	8.383	1.00	13.14	A
ATOM	92	N	VAL	126	-10.550	-9.129	6.806	1.00	15.65	A
ATOM	93	H	VAL	126	-10.169	-8.286	6.428	1.00	15.00	A
ATOM	94	CA	VAL	126	-11.743	-9.717	6.201	1.00	14.38	A
ATOM	95	CB	VAL	126	-12.877	-8.808	6.675	1.00	13.37	A
ATOM	96	CG1	VAL	126	-13.794	-9.722	7.379	1.00	12.60	A
ATOM	97	CG2	VAL	126	-13.449	-7.663	5.814	1.00	9.61	A
ATOM	98	C	VAL	126	-11.502	-9.971	4.685	1.00	16.03	A
ATOM	99	O	VAL	126	-10.684	-9.297	4.074	1.00	16.42	A
ATOM	100	N	ILE	127	-12.118	-11.013	4.136	1.00	15.99	A
ATOM	101	H	ILE	127	-12.807	-11.481	4.691	1.00	15.00	A
ATOM	102	CA	ILE	127	-11.651	-11.532	2.831	1.00	14.86	A
ATOM	103	CB	ILE	127	-11.414	-13.051	3.002	1.00	17.56	A
ATOM	104	CG2	ILE	127	-11.716	-13.910	1.765	1.00	17.17	A
ATOM	105	CG1	ILE	127	-9.972	-13.316	3.399	1.00	16.47	A
ATOM	106	CD1	ILE	127	-9.705	-12.992	4.864	1.00	19.64	A
ATOM	107	C	ILE	127	-12.691	-11.269	1.765	1.00	18.96	A
ATOM	108	O	ILE	127	-13.898	-11.391	2.016	1.00	20.01	A
ATOM	109	N	SER	128	-12.229	-10.882	0.581	1.00	17.54	A
ATOM	110	H	SER	128	-11.232	-10.871	0.382	1.00	15.00	A
ATOM	111	CA	SER	128	-13.274	-10.667	-0.437	1.00	15.55	A
ATOM	112	CB	SER	128	-12.664	-10.130	-1.706	1.00	18.16	A
ATOM	113	OG	SER	128	-12.205	-11.207	-2.574	1.00	19.90	A
ATOM	114	HG	SER	128	-11.832	-11.931	-2.029	1.00	15.00	A
ATOM	115	C	SER	128	-14.295	-11.761	-0.792	1.00	13.62	A
ATOM	116	O	SER	128	-14.052	-12.960	-0.832	1.00	8.98	A
ATOM	117	N	GLU	129	-15.492	-11.246	-1.027	1.00	13.36	A
ATOM	118	H	GLU	129	-15.661	-10.257	-0.937	1.00	15.00	A
ATOM	119	CA	GLU	129	-16.379	-12.024	-1.840	1.00	17.20	A

FIGURE 17C

ATOM	120	CB	GLU	129	-17.052	-13.117	-1.021	1.00	21.55	A
ATOM	121	CG	GLU	129	-18.092	-12.694	-0.036	1.00	17.92	A
ATOM	122	CD	GLU	129	-18.781	-13.951	0.376	1.00	21.88	A
ATOM	123	OE1	GLU	129	-19.997	-13.932	0.368	1.00	32.23	A
ATOM	124	OE2	GLU	129	-18.150	-14.938	0.734	1.00	33.12	A
ATOM	125	C	GLU	129	-17.371	-11.409	-2.809	1.00	17.71	A
ATOM	126	O	GLU	129	-17.972	-10.389	-2.553	1.00	21.59	A
ATOM	127	N	ALA	130	-17.550	-12.145	-3.914	1.00	20.52	A
ATOM	128	H	ALA	130	-17.136	-13.057	-3.923	1.00	15.00	A
ATOM	129	CA	ALA	130	-18.379	-11.649	-5.019	1.00	23.36	A
ATOM	130	CB	ALA	130	-18.424	-12.633	-6.208	1.00	19.66	A
ATOM	131	C	ALA	130	-19.811	-11.298	-4.570	1.00	26.86	A
ATOM	132	O	ALA	130	-20.519	-12.022	-3.869	1.00	29.40	A
ATOM	133	N	SER	131	-20.198	-10.086	-4.968	1.00	21.70	A
ATOM	134	H	SER	131	-19.515	-9.481	-5.410	1.00	15.00	A
ATOM	135	CA	SER	131	-21.592	-9.782	-4.732	1.00	20.04	A
ATOM	136	CB	SER	131	-21.829	-8.266	-4.787	1.00	20.65	A
ATOM	137	OG	SER	131	-23.182	-8.001	-4.435	1.00	15.24	A
ATOM	138	HG	SER	131	-23.329	-7.069	-4.559	1.00	15.00	A
ATOM	139	C	SER	131	-22.546	-10.501	-5.668	1.00	17.15	A
ATOM	140	O	SER	131	-22.236	-10.853	-6.786	1.00	14.30	A
ATOM	141	N	SER	132	-23.756	-10.731	-5.187	1.00	20.15	A
ATOM	142	H	SER	132	-23.967	-10.586	-4.209	1.00	15.00	A
ATOM	143	CA	SER	132	-24.674	-11.250	-6.218	1.00	21.62	A
ATOM	144	CB	SER	132	-25.266	-12.616	-5.893	1.00	16.00	A
ATOM	145	OG	SER	132	-26.203	-12.324	-4.894	1.00	23.84	A
ATOM	146	HG	SER	132	-26.016	-12.944	-4.179	1.00	15.00	A
ATOM	147	C	SER	132	-25.727	-10.268	-6.671	1.00	20.07	A
ATOM	148	O	SER	132	-26.535	-10.544	-7.547	1.00	20.27	A
ATOM	149	N	LYS	133	-25.606	-9.063	-6.118	1.00	21.87	A
ATOM	150	H	LYS	133	-24.904	-8.969	-5.397	1.00	15.00	A
ATOM	151	CA	LYS	133	-26.406	-7.916	-6.517	1.00	19.23	A
ATOM	152	CB	LYS	133	-27.024	-7.309	-5.256	1.00	23.08	A
ATOM	153	CG	LYS	133	-27.684	-8.364	-4.354	1.00	21.07	A
ATOM	154	CD	LYS	133	-29.174	-8.110	-4.320	1.00	27.36	A
ATOM	155	CE	LYS	133	-29.939	-7.884	-5.670	1.00	30.56	A
ATOM	156	NZ	LYS	133	-31.323	-7.515	-5.345	1.00	21.56	A
ATOM	157	HZ1	LYS	133	-31.862	-7.351	-6.218	1.00	15.00	A
ATOM	158	HZ2	LYS	133	-31.753	-8.299	-4.811	1.00	15.00	A
ATOM	159	HZ3	LYS	133	-31.333	-6.654	-4.760	1.00	15.00	A
ATOM	160	C	LYS	133	-25.579	-6.876	-7.194	1.00	20.10	A
ATOM	161	O	LYS	133	-24.378	-6.801	-7.007	1.00	17.94	A
ATOM	162	N	THR	134	-26.260	-6.052	-7.983	1.00	22.95	A
ATOM	163	H	THR	134	-27.275	-6.130	-8.036	1.00	15.00	A
ATOM	164	CA	THR	134	-25.556	-4.879	-8.561	1.00	27.89	A
ATOM	165	CB	THR	134	-26.498	-4.274	-9.592	1.00	24.59	A
ATOM	166	OG1	THR	134	-26.540	-5.037	-10.792	1.00	24.32	A
ATOM	167	HG1	THR	134	-26.232	-4.411	-11.456	1.00	15.00	A
ATOM	168	CG2	THR	134	-26.044	-2.897	-9.968	1.00	22.97	A
ATOM	169	C	THR	134	-24.987	-3.798	-7.559	1.00	32.51	A
ATOM	170	O	THR	134	-25.659	-3.461	-6.603	1.00	38.43	A
ATOM	171	N	THR	135	-23.717	-3.352	-7.690	1.00	35.98	A
ATOM	172	H	THR	135	-23.292	-3.555	-8.585	1.00	15.00	A
ATOM	173	CA	THR	135	-22.964	-3.469	-6.386	1.00	36.02	A
ATOM	174	CB	THR	135	-21.575	-4.276	-6.534	1.00	36.01	A
ATOM	175	OG1	THR	135	-21.645	-5.388	-7.488	1.00	30.60	A
ATOM	176	HG1	THR	135	-22.255	-6.094	-7.312	1.00	15.00	A
ATOM	177	CG2	THR	135	-20.866	-4.776	-5.264	1.00	35.55	A
ATOM	178	C	THR	135	-22.949	-2.266	-5.404	1.00	30.25	A
ATOM	179	O	THR	135	-23.541	-2.348	-4.331	1.00	28.35	A

FIGURE 17D

ATOM	180	N	SER	136	-22.294	-1.146	-5.776	1.00	23.29	A
ATOM	181	H	SER	136	-22.828	-0.357	-5.460	1.00	15.00	A
ATOM	182	CA	SER	136	-20.857	-1.051	-6.143	1.00	23.04	A
ATOM	183	CB	SER	136	-20.560	0.187	-6.965	1.00	21.03	A
ATOM	184	OG	SER	136	-20.624	1.261	-6.043	1.00	28.21	A
ATOM	185	HG	SER	136	-19.815	1.793	-6.008	1.00	15.00	A
ATOM	186	C	SER	136	-19.853	-1.090	-4.958	1.00	21.77	A
ATOM	187	O	SER	136	-18.630	-1.096	-5.080	1.00	21.94	A
ATOM	188	N	VAL	137	-20.452	-1.227	-3.752	1.00	24.03	A
ATOM	189	H	VAL	137	-21.440	-1.063	-3.705	1.00	15.00	A
ATOM	190	CA	VAL	137	-19.699	-1.632	-2.570	1.00	19.65	A
ATOM	191	CB	VAL	137	-20.218	-1.010	-1.248	1.00	21.14	A
ATOM	192	CG1	VAL	137	-20.419	-1.907	-0.058	1.00	18.16	A
ATOM	193	CG2	VAL	137	-21.322	-0.026	-1.442	1.00	13.49	A
ATOM	194	C	VAL	137	-19.370	-3.116	-2.473	1.00	17.15	A
ATOM	195	O	VAL	137	-20.209	-3.969	-2.593	1.00	16.69	A
ATOM	196	N	LEU	138	-18.077	-3.344	-2.271	1.00	15.84	A
ATOM	197	H	LEU	138	-17.502	-2.528	-2.246	1.00	15.00	A
ATOM	198	CA	LEU	138	-17.507	-4.667	-1.938	1.00	18.21	A
ATOM	199	CB	LEU	138	-15.962	-4.530	-1.791	1.00	13.60	A
ATOM	200	CG	LEU	138	-15.273	-3.854	-2.998	1.00	16.09	A
ATOM	201	CD1	LEU	138	-15.923	-4.379	-4.300	1.00	20.35	A
ATOM	202	CD2	LEU	138	-13.710	-3.936	-2.982	1.00	12.34	A
ATOM	203	C	LEU	138	-18.170	-5.480	-0.772	1.00	16.29	A
ATOM	204	O	LEU	138	-18.498	-4.986	0.301	1.00	12.97	A
ATOM	205	N	GLN	139	-18.345	-6.768	-1.035	1.00	13.04	A
ATOM	206	H	GLN	139	-18.052	-7.078	-1.960	1.00	15.00	A
ATOM	207	CA	GLN	139	-18.757	-7.658	0.013	1.00	15.32	A
ATOM	208	CB	GLN	139	-19.847	-8.678	-0.481	1.00	13.99	A
ATOM	209	CG	GLN	139	-21.068	-7.960	-1.113	1.00	20.85	A
ATOM	210	CD	GLN	139	-21.872	-7.022	-0.193	1.00	22.04	A
ATOM	211	OE1	GLN	139	-22.343	-7.439	0.878	1.00	25.45	A
ATOM	212	NE2	GLN	139	-21.963	-5.739	-0.618	1.00	17.74	A
ATOM	213	HE21	GLN	139	-22.697	-5.181	-0.206	1.00	15.00	A
ATOM	214	HE22	GLN	139	-21.460	-5.326	-1.374	1.00	15.00	A
ATOM	215	C	GLN	139	-17.527	-8.383	0.541	1.00	14.26	A
ATOM	216	O	GLN	139	-16.554	-8.640	-0.144	1.00	14.40	A
ATOM	217	N	TRP	140	-17.647	-8.780	1.805	1.00	12.80	A
ATOM	218	H	TRP	140	-18.433	-8.447	2.297	1.00	15.00	A
ATOM	219	CA	TRP	140	-16.542	-9.500	2.463	1.00	14.03	A
ATOM	220	CB	TRP	140	-15.813	-8.623	3.483	1.00	14.18	A
ATOM	221	CG	TRP	140	-15.467	-7.291	2.823	1.00	8.44	A
ATOM	222	CD2	TRP	140	-14.379	-6.966	1.941	1.00	9.01	A
ATOM	223	CE2	TRP	140	-14.549	-5.625	1.482	1.00	8.40	A
ATOM	224	CE3	TRP	140	-13.215	-7.688	1.581	1.00	10.14	A
ATOM	225	CD1	TRP	140	-16.225	-6.137	2.863	1.00	11.29	A
ATOM	226	NE1	TRP	140	-15.710	-5.150	2.077	1.00	14.27	A
ATOM	227	HE1	TRP	140	-16.121	-4.268	2.010	1.00	15.00	A
ATOM	228	CZ2	TRP	140	-13.640	-5.009	0.590	1.00	8.16	A
ATOM	229	CZ3	TRP	140	-12.292	-7.069	0.713	1.00	13.90	A
ATOM	230	CH2	TRP	140	-12.497	-5.749	0.215	1.00	12.11	A
ATOM	231	C	TRP	140	-17.016	-10.701	3.170	1.00	14.34	A
ATOM	232	O	TRP	140	-18.193	-10.862	3.392	1.00	16.00	A
ATOM	233	N	ALA	141	-16.082	-11.528	3.558	1.00	14.80	A
ATOM	234	H	ALA	141	-15.133	-11.377	3.294	1.00	15.00	A
ATOM	235	CA	ALA	141	-16.489	-12.617	4.394	1.00	15.27	A
ATOM	236	CB	ALA	141	-16.504	-13.920	3.583	1.00	16.97	A
ATOM	237	C	ALA	141	-15.585	-12.761	5.607	1.00	15.90	A
ATOM	238	O	ALA	141	-14.453	-12.338	5.550	1.00	14.25	A
ATOM	239	N	GLU	142	-16.068	-13.366	6.688	1.00	19.74	A

FIGURE 17E

ATOM	240	H	GLU	142	-17.055	-13.574	6.688	1.00	15.00	A
ATOM	241	CA	GLU	142	-15.149	-13.759	7.731	1.00	25.93	A
ATOM	242	CB	GLU	142	-15.794	-13.910	9.117	1.00	21.75	A
ATOM	243	CG	GLU	142	-15.716	-12.456	9.647	1.00	24.05	A
ATOM	244	CD	GLU	142	-16.749	-12.087	10.711	1.00	26.61	A
ATOM	245	OE1	GLU	142	-17.908	-11.888	10.361	1.00	34.72	A
ATOM	246	OE2	GLU	142	-16.404	-11.984	11.886	1.00	30.07	A
ATOM	247	C	GLU	142	-14.200	-14.797	7.193	1.00	33.25	A
ATOM	248	O	GLU	142	-13.156	-14.349	6.737	1.00	41.84	A
ATOM	249	N	LYS	143	-14.577	-16.080	7.084	1.00	34.17	A
ATOM	250	H	LYS	143	-15.432	-16.384	7.492	1.00	15.00	A
ATOM	251	CA	LYS	143	-13.882	-16.854	5.980	1.00	35.31	A
ATOM	252	CB	LYS	143	-14.673	-16.603	4.681	1.00	37.64	A
ATOM	253	CG	LYS	143	-14.300	-17.505	3.531	1.00	47.37	A
ATOM	254	CD	LYS	143	-15.022	-17.284	2.202	1.00	50.37	A
ATOM	255	CE	LYS	143	-14.686	-16.047	1.357	1.00	49.23	A
ATOM	256	NZ	LYS	143	-15.632	-16.097	0.221	1.00	51.67	A
ATOM	257	HZ1	LYS	143	-15.333	-15.445	-0.534	1.00	15.00	A
ATOM	258	HZ2	LYS	143	-15.680	-17.061	-0.177	1.00	15.00	A
ATOM	259	HZ3	LYS	143	-16.564	-15.833	0.585	1.00	15.00	A
ATOM	260	C	LYS	143	-12.330	-16.979	5.637	1.00	32.80	A
ATOM	261	O	LYS	143	-11.831	-18.041	5.276	1.00	35.64	A
ATOM	262	N	GLY	144	-11.522	-15.923	5.637	1.00	28.26	A
ATOM	263	H	GLY	144	-11.718	-14.995	5.910	1.00	15.00	A
ATOM	264	CA	GLY	144	-10.243	-16.458	5.194	1.00	32.94	A
ATOM	265	C	GLY	144	-9.178	-16.862	6.180	1.00	29.93	A
ATOM	266	O	GLY	144	-9.345	-17.454	7.205	1.00	24.67	A
ATOM	267	N	TYR	145	-8.069	-16.270	5.815	1.00	26.37	A
ATOM	268	H	TYR	145	-8.160	-15.729	4.966	1.00	15.00	A
ATOM	269	CA	TYR	145	-7.027	-16.002	6.777	1.00	27.61	A
ATOM	270	CB	TYR	145	-5.708	-15.877	5.947	1.00	37.54	A
ATOM	271	CG	TYR	145	-5.962	-15.774	4.456	1.00	50.95	A
ATOM	272	CD1	TYR	145	-5.682	-14.633	3.706	1.00	53.22	A
ATOM	273	CE1	TYR	145	-6.313	-14.377	2.468	1.00	60.28	A
ATOM	274	CD2	TYR	145	-6.591	-16.847	3.791	1.00	53.11	A
ATOM	275	CE2	TYR	145	-7.207	-16.699	2.551	1.00	56.30	A
ATOM	276	CZ	TYR	145	-7.162	-15.430	1.873	1.00	61.12	A
ATOM	277	OH	TYR	145	-7.812	-15.119	0.665	1.00	62.63	A
ATOM	278	HH	TYR	145	-8.575	-15.686	0.401	1.00	15.00	A
ATOM	279	C	TYR	145	-7.532	-14.762	7.620	1.00	22.41	A
ATOM	280	C	TYR	145	-7.000	-13.677	7.650	1.00	22.68	A
ATOM	281	N	TYR	146	-8.731	-14.884	8.196	1.00	20.39	A
ATOM	282	H	TYR	146	-8.935	-15.824	8.509	1.00	15.00	A
ATOM	283	CA	TYR	146	-9.423	-13.700	8.725	1.00	20.40	A
ATOM	284	CB	TYR	146	-10.886	-13.673	8.306	1.00	22.53	A
ATOM	285	CG	TYR	146	-11.710	-14.460	9.286	1.00	23.02	A
ATOM	286	CD1	TYR	146	-11.635	-15.873	9.236	1.00	26.99	A
ATOM	287	CE1	TYR	146	-12.254	-16.623	10.239	1.00	25.44	A
ATOM	288	CD2	TYR	146	-12.477	-13.766	10.236	1.00	23.45	A
ATOM	289	CE2	TYR	146	-13.150	-14.520	11.205	1.00	26.81	A
ATOM	290	CZ	TYR	146	-13.007	-15.937	11.204	1.00	27.40	A
ATOM	291	OH	TYR	146	-13.647	-16.689	12.170	1.00	31.91	A
ATOM	292	HH	TYR	146	-12.911	-17.080	12.676	1.00	15.00	A
ATOM	293	C	TYR	146	-9.291	-13.419	10.219	1.00	18.79	A
ATOM	294	O	TYR	146	-8.904	-14.232	11.012	1.00	16.13	A
ATOM	295	N	THR	147	-9.596	-12.169	10.556	1.00	17.54	A
ATOM	296	H	THR	147	-9.973	-11.607	9.830	1.00	15.00	A
ATOM	297	CA	THR	147	-9.432	-11.764	11.948	1.00	14.06	A
ATOM	298	CB	THR	147	-8.162	-10.875	12.182	1.00	13.66	A
ATOM	299	OG1	THR	147	-6.912	-11.505	11.856	1.00	12.56	A

FIGURE 17F

ATOM	300	HG1	THR	147	-6.934	-11.898	10.980	1.00	15.00	A
ATOM	301	OG2	THR	147	-8.025	-10.236	13.554	1.00	17.22	A
ATOM	302	C	THR	147	-10.619	-10.925	12.253	1.00	15.60	A
ATOM	303	O	THR	147	-11.044	-10.074	11.496	1.00	18.39	A
ATOM	304	N	MET	148	-11.144	-11.139	13.412	1.00	20.67	A
ATOM	305	H	MET	148	-10.838	-11.988	13.828	1.00	15.00	A
ATOM	306	CA	MET	148	-12.124	-10.311	14.110	1.00	19.71	A
ATOM	307	CB	MET	148	-13.546	-10.702	13.705	1.00	17.89	A
ATOM	308	CG	MET	148	-14.541	-9.580	14.019	1.00	13.53	A
ATOM	309	SD	MET	148	-14.492	-8.149	12.952	1.00	14.69	A
ATOM	310	CE	MET	148	-14.566	-8.928	11.333	1.00	10.10	A
ATOM	311	C	MET	148	-11.915	-10.282	15.639	1.00	21.49	A
ATOM	312	O	MET	148	-12.594	-10.905	16.436	1.00	22.98	A
ATOM	313	N	SER	149	-10.955	-9.412	16.055	1.00	20.58	A
ATOM	314	H	SER	149	-10.516	-8.786	15.406	1.00	15.00	A
ATOM	315	CA	SER	149	-10.388	-9.698	17.419	1.00	19.11	A
ATOM	316	CB	SER	149	-9.174	-8.860	17.792	1.00	12.17	A
ATOM	317	OG	SER	149	-9.540	-7.513	17.975	1.00	14.10	A
ATOM	318	HG	SER	149	-9.571	-7.487	18.934	1.00	15.00	A
ATOM	319	C	SER	149	-11.203	-9.844	18.727	1.00	22.19	A
ATOM	320	O	SER	149	-10.728	-10.267	19.772	1.00	22.95	A
ATOM	321	N	ASN	150	-12.456	-9.322	18.631	1.00	22.71	A
ATOM	322	H	ASN	150	-12.782	-9.247	17.688	1.00	15.00	A
ATOM	323	CA	ASN	150	-13.361	-9.236	19.764	1.00	20.32	A
ATOM	324	CB	ASN	150	-12.734	-8.446	20.955	1.00	21.56	A
ATOM	325	CG	ASN	150	-12.343	-6.962	20.706	1.00	20.71	A
ATOM	326	OD1	ASN	150	-13.059	-6.187	20.119	1.00	17.81	A
ATOM	327	ND2	ASN	150	-11.222	-6.485	21.271	1.00	23.86	A
ATOM	328	HD21	ASN	150	-11.035	-5.521	21.092	1.00	15.00	A
ATOM	329	HD22	ASN	150	-10.670	-7.109	21.821	1.00	15.00	A
ATOM	330	C	ASN	150	-14.644	-8.657	19.256	1.00	20.60	A
ATOM	331	O	ASN	150	-14.718	-8.130	18.148	1.00	20.56	A
ATOM	332	N	ASN	151	-15.637	-8.713	20.149	1.00	23.49	A
ATOM	333	H	ASN	151	-15.455	-9.124	21.038	1.00	15.00	A
ATOM	334	CA	ASN	151	-16.974	-8.080	19.823	1.00	24.71	A
ATOM	335	CB	ASN	151	-18.130	-8.645	20.712	1.00	28.30	A
ATOM	336	CG	ASN	151	-17.959	-8.271	22.173	1.00	33.23	A
ATOM	337	OD1	ASN	151	-17.075	-7.562	22.606	1.00	39.79	A
ATOM	338	ND2	ASN	151	-18.782	-8.838	23.011	1.00	38.32	A
ATOM	339	HD21	ASN	151	-18.553	-8.524	23.928	1.00	15.00	A
ATOM	340	HD22	ASN	151	-19.495	-9.465	22.733	1.00	15.00	A
ATOM	341	C	ASN	151	-17.172	-6.531	19.645	1.00	22.53	A
ATOM	342	O	ASN	151	-18.254	-6.048	19.374	1.00	21.32	A
ATOM	343	N	LEU	152	-16.066	-5.762	19.859	1.00	23.00	A
ATOM	344	H	LEU	152	-15.247	-6.289	20.070	1.00	15.00	A
ATOM	345	CA	LEU	152	-15.924	-4.335	19.525	1.00	18.87	A
ATOM	346	CB	LEU	152	-14.830	-3.700	20.325	1.00	21.77	A
ATOM	347	CG	LEU	152	-14.981	-3.999	21.806	1.00	24.80	A
ATOM	348	CD1	LEU	152	-16.390	-3.645	22.316	1.00	22.82	A
ATOM	349	CD2	LEU	152	-13.847	-3.256	22.556	1.00	23.56	A
ATOM	350	C	LEU	152	-15.565	-3.993	18.094	1.00	17.34	A
ATOM	351	O	LEU	152	-15.590	-2.840	17.708	1.00	13.39	A
ATOM	352	N	VAL	153	-15.267	-5.054	17.309	1.00	18.65	A
ATOM	353	H	VAL	153	-15.156	-5.962	17.716	1.00	15.00	A
ATOM	354	CA	VAL	153	-15.439	-4.910	15.849	1.00	16.81	A
ATOM	355	CB	VAL	153	-14.138	-5.021	14.980	1.00	15.33	A
ATOM	356	CG1	VAL	153	-12.908	-5.718	15.562	1.00	21.22	A
ATOM	357	CG2	VAL	153	-13.775	-3.757	14.287	1.00	16.95	A
ATOM	358	C	VAL	153	-16.405	-5.964	15.301	1.00	13.48	A
ATOM	359	O	VAL	153	-16.363	-7.116	15.647	1.00	13.06	A

FIGURE 17G

ATOM	360	N	THR	154	-17.207	-5.546	14.358	1.00	12.06	A
ATOM	361	H	THR	154	-17.313	-4.568	14.215	1.00	15.00	A
ATOM	362	CA	THR	154	-17.903	-6.600	13.615	1.00	16.26	A
ATOM	363	CB	THR	154	-19.366	-6.747	14.157	1.00	19.51	A
ATOM	364	OG1	THR	154	-19.995	-5.459	14.205	1.00	19.31	A
ATOM	365	HG1	THR	154	-20.577	-5.508	14.949	1.00	15.00	A
ATOM	366	CG2	THR	154	-19.502	-7.288	15.571	1.00	21.62	A
ATOM	367	C	THR	154	-17.997	-6.252	12.107	1.00	18.12	A
ATOM	368	O	THR	154	-17.952	-5.110	11.605	1.00	16.55	A
ATOM	369	N	LEU	155	-18.101	-7.324	11.357	1.00	16.77	A
ATOM	370	H	LEU	155	-18.056	-8.202	11.791	1.00	15.00	A
ATOM	371	CA	LEU	155	-18.514	-7.198	9.967	1.00	17.10	A
ATOM	372	CB	LEU	155	-17.829	-8.353	9.204	1.00	20.04	A
ATOM	373	CG	LEU	155	-17.524	-8.428	7.692	1.00	20.81	A
ATOM	374	CD1	LEU	155	-17.822	-7.159	6.908	1.00	17.03	A
ATOM	375	CD2	LEU	155	-17.912	-9.810	7.139	1.00	12.42	A
ATOM	376	C	LEU	155	-20.055	-7.187	9.904	1.00	20.71	A
ATOM	377	O	LEU	155	-20.712	-8.163	10.217	1.00	18.01	A
ATOM	378	N	GLU	156	-20.593	-5.995	9.561	1.00	19.51	A
ATOM	379	H	GLU	156	-19.959	-5.230	9.440	1.00	15.00	A
ATOM	380	CA	GLU	156	-22.036	-5.888	9.413	1.00	21.95	A
ATOM	381	CB	GLU	156	-22.641	-4.631	10.033	1.00	18.95	A
ATOM	382	CG	GLU	156	-22.098	-4.412	11.436	1.00	27.68	A
ATOM	383	CD	GLU	155	-22.721	-5.194	12.587	1.00	31.62	A
ATOM	384	OE1	GLU	156	-23.347	-6.248	12.367	1.00	33.40	A
ATOM	385	OE2	GLU	156	-22.532	-4.721	13.724	1.00	35.00	A
ATOM	386	C	GLU	156	-22.457	-5.966	7.964	1.00	25.36	A
ATOM	387	O	GLU	156	-21.958	-5.298	7.077	1.00	22.70	A
ATOM	388	N	ASN	157	-23.437	-6.808	7.696	1.00	30.92	A
ATOM	389	H	ASN	157	-23.594	-7.590	8.300	1.00	15.00	A
ATOM	390	CA	ASN	157	-23.804	-6.620	6.300	1.00	33.31	A
ATOM	391	CB	ASN	157	-23.856	-7.970	5.614	1.00	31.69	A
ATOM	392	CG	ASN	157	-23.669	-7.693	4.168	1.00	27.70	A
ATOM	393	OD1	ASN	157	-23.397	-6.593	3.810	1.00	25.89	A
ATOM	394	ND2	ASN	157	-23.893	-8.640	3.275	1.00	41.69	A
ATOM	395	HD21	ASN	157	-24.069	-9.603	3.467	1.00	15.00	A
ATOM	396	HD22	ASN	157	-23.745	-8.295	2.340	1.00	15.00	A
ATOM	397	C	ASN	157	-24.988	-5.658	6.118	1.00	35.08	A
ATOM	398	O	ASN	157	-26.107	-5.949	6.499	1.00	37.06	A
ATOM	399	N	GLY	158	-24.746	-4.443	5.560	1.00	40.03	A
ATOM	400	H	GLY	158	-25.601	-3.952	5.429	1.00	15.00	A
ATOM	401	CA	GLY	158	-23.422	-3.887	5.121	1.00	38.11	A
ATOM	402	C	GLY	158	-23 062	-3.720	3.617	1.00	37.48	A
ATOM	403	O	GLY	158	-23.890	-3.108	2.950	1.00	41.11	A
ATOM	404	N	LYS	159	-21.867	-4.220	3.135	1.00	32.75	A
ATOM	405	H	LYS	159	-21.904	-4.134	2.130	1.00	15.00	A
ATOM	406	CA	LYS	159	-20.828	-4.928	3.962	1.00	27.83	A
ATOM	407	CB	LYS	159	-20.317	-6.122	3.217	1.00	28.17	A
ATOM	408	CG	LYS	159	-19.734	-7.168	4.069	1.00	20.48	A
ATOM	409	CD	LYS	159	-20.533	-8.426	4.192	1.00	29.61	A
ATOM	410	CE	LYS	159	-20.577	-9.191	2.869	1.00	40.41	A
ATOM	411	NZ	LYS	159	-20.796	-10.663	2.986	1.00	40.88	A
ATOM	412	HZ1	LYS	159	-20.739	-11.087	2.035	1.00	15.00	A
ATOM	413	HZ2	LYS	159	-20.070	-11.087	3.600	1.00	15.00	A
ATOM	414	HZ3	LYS	159	-21.738	-10.848	3.389	1.00	15.00	A
ATOM	415	C	LYS	159	-19.688	-4.065	4.463	1.00	26.08	A
ATOM	416	O	LYS	159	-19.023	-3.369	3.696	1.00	28.01	A
ATOM	417	N	GLN	160	-19.663	-3.990	5.807	1.00	18.90	A
ATOM	418	H	GLN	160	-20.211	-4.674	6.379	1.00	15.00	A
ATOM	419	CA	GLN	160	-18.922	-2.939	6.464	1.00	13.89	A

FIGURE 17H

ATOM	420	CB	GLN	160	-19.778	-1.694	6.611	1.00	16.79	A
ATOM	421	CG	GLN	160	-20.881	-1.896	7.633	1.00	18.34	A
ATOM	422	CD	GLN	160	-22.133	-1.166	7.193	1.00	23.97	A
ATOM	423	OE1	GLN	160	-23.086	-0.970	7.893	1.00	31.18	A
ATOM	424	NE2	GLN	160	-22.257	-0.771	5.948	1.00	28.16	A
ATOM	425	HE21	GLN	160	-23.194	-0.420	5.928	1.00	15.00	A
ATOM	426	HE22	GLN	160	-21.624	-0.780	5.186	1.00	15.00	A
ATOM	427	C	GLN	160	-18.313	-3.309	7.777	1.00	12.87	A
ATOM	428	O	GLN	160	-18.838	-4.151	8.498	1.00	14.78	A
ATOM	429	N	LEU	161	-17.187	-2.637	8.085	1.00	11.22	A
ATOM	430	H	LEU	161	-16.767	-2.124	7.340	1.00	15.00	A
ATOM	431	CA	LEU	161	-16.583	-2.870	9.405	1.00	9.71	A
ATOM	432	CB	LEU	161	-15.052	-2.939	9.390	1.00	4.67	A
ATOM	433	CG	LEU	161	-14.438	-4.060	8.559	1.00	7.30	A
ATOM	434	CD1	LEU	161	-14.511	-5.447	9.207	1.00	10.80	A
ATOM	435	CD2	LEU	161	-12.964	-3.794	8.389	1.00	5.48	A
ATOM	436	C	LEU	161	-17.082	-1.836	10.412	1.00	10.17	A
ATOM	437	O	LEU	161	-16.826	-0.657	10.341	1.00	13.36	A
ATOM	438	N	THR	162	-17.848	-2.338	11.375	1.00	16.94	A
ATOM	439	H	THR	162	-18.153	-3.279	11.251	1.00	15.00	A
ATOM	440	CA	THR	162	-18.317	-1.480	12.493	1.00	16.14	A
ATOM	441	CB	THR	162	-19.807	-1.769	12.640	1.00	13.33	A
ATOM	442	OG1	THR	162	-20.339	-1.707	11.308	1.00	16.73	A
ATOM	443	HG1	THR	162	-21.211	-1.254	11.343	1.00	15.00	A
ATOM	444	CG2	THR	162	-20.553	-0.832	13.562	1.00	15.01	A
ATOM	445	C	THR	162	-17.531	-1.547	13.842	1.00	13.28	A
ATOM	446	O	THR	162	-17.358	-2.587	14.449	1.00	20.21	A
ATOM	447	N	VAL	163	-16.994	-0.437	14.282	1.00	14.22	A
ATOM	448	H	VAL	163	-16.859	0.243	13.567	1.00	15.00	A
ATOM	449	CA	VAL	163	-16.326	-0.358	15.586	1.00	15.72	A
ATOM	450	CB	VAL	163	-15.038	0.426	15.428	1.00	11.82	A
ATOM	451	CG1	VAL	163	-15.191	1.944	15.368	1.00	9.87	A
ATOM	452	CG2	VAL	163	-14.229	-0.124	14.245	1.00	18.88	A
ATOM	453	C	VAL	163	-17.193	0.283	16.706	1.00	17.93	A
ATOM	454	O	VAL	163	-18.001	1.180	16.453	1.00	20.25	A
ATOM	455	N	LYS	164	-17.037	-0.232	17.925	1.00	15.44	A
ATOM	456	H	LYS	164	-16.254	-0.858	18.020	1.00	15.00	A
ATOM	457	CA	LYS	164	-17.856	0.138	19.109	1.00	17.33	A
ATOM	458	CB	LYS	164	-18.351	-1.150	19.807	1.00	19.58	A
ATOM	459	CG	LYS	164	-19.214	-1.885	18.759	1.00	23.56	A
ATOM	460	CD	LYS	164	-19.417	-3.410	18.851	1.00	28.85	A
ATOM	461	CE	LYS	164	-20.039	-4.047	17.554	1.00	33.81	A
ATOM	462	NZ	LYS	164	-19.428	-3.681	16.227	1.00	18.98	A
ATOM	463	HZ1	LYS	164	-19.195	-2.667	16.222	1.00	15.00	A
ATOM	464	HZ2	LYS	164	-18.552	-4.223	16.092	1.00	15.00	A
ATOM	465	HZ3	LYS	164	-20.084	-3.888	15.445	1.00	15.00	A
ATOM	466	C	LYS	164	-17.193	1.099	20.056	1.00	15.14	A
ATOM	467	O	LYS	164	-17.712	1.588	21.048	1.00	17.72	A
ATOM	468	N	ARG	165	-15.992	1.428	19.621	1.00	17.49	A
ATOM	469	H	ARG	165	-15.550	0.838	18.932	1.00	15.00	A
ATOM	470	CA	ARG	165	-15.184	2.415	20.325	1.00	20.18	A
ATOM	471	CB	ARG	165	-13.985	1.806	21.049	1.00	24.65	A
ATOM	472	CG	ARG	165	-14.363	0.833	22.126	1.00	29.54	A
ATOM	473	CD	ARG	165	-13.274	1.077	23.145	1.00	38.82	A
ATOM	474	NE	ARG	165	-13.719	1.998	24.186	1.00	43.41	A
ATOM	475	HE	ARG	165	-14.331	1.671	24.908	1.00	15.00	A
ATOM	476	CZ	ARG	165	-13.190	3.250	24.362	1.00	44.06	A
ATOM	477	NH1	ARG	165	-13.406	3.765	25.562	1.00	41.25	A
ATOM	478	HH11	ARG	165	-13.054	4.683	25.763	1.00	15.00	A
ATOM	479	HH12	ARG	165	-13.919	3.249	26.250	1.00	15.00	A

FIGURE 17I

ATOM	480	NH2	ARG	165	-12.485	3.946	23.425	1.00	31.65	A
ATOM	481	HH21	ARG	165	-12.133	4.860	23.623	1.00	15.00	A
ATOM	482	HH22	ARG	165	-12.322	3.527	22.530	1.00	15.00	A
ATOM	483	C	ARG	165	-14.608	3.554	19.510	1.00	17.70	A
ATOM	484	O	ARG	165	-14.018	3.450	18.441	1.00	18.26	A
ATOM	485	N	GLN	166	-14.763	4.687	20.151	1.00	17.43	A
ATOM	486	H	GLN	166	-15.263	4.614	21.007	1.00	15.00	A
ATOM	487	CA	GLN	166	-14.138	5.911	19.698	1.00	19.00	A
ATOM	488	CB	GLN	166	-14.613	7.021	20.610	1.00	23.79	A
ATOM	489	CG	GLN	166	-14.067	8.409	20.386	1.00	34.06	A
ATOM	490	CD	GLN	166	-15.178	9.399	20.659	1.00	45.91	A
ATOM	491	OE1	GLN	166	-15.102	10.492	20.135	1.00	53.64	A
ATOM	492	NE2	GLN	166	-16.202	9.046	21.418	1.00	44.10	A
ATOM	493	HE21	GLN	166	-16.906	9.765	21.443	1.00	15.00	A
ATOM	494	HE22	GLN	166	-16.577	8.287	21.935	1.00	15.00	A
ATOM	495	C	GLN	166	-12.649	5.881	19.644	1.00	17.48	A
ATOM	496	O	GLN	166	-12.029	5.378	20.561	1.00	18.13	A
ATOM	497	N	GLY	167	-12.160	6.478	18.565	1.00	14.83	A
ATOM	498	H	GLY	167	-12.750	6.836	17.850	1.00	15.00	A
ATOM	499	CA	GLY	167	-10.728	6.711	18.557	1.00	16.28	A
ATOM	500	C	GLY	167	-10.044	6.685	17.204	1.00	16.48	A
ATOM	501	O	GLY	167	-10.674	6.601	16.162	1.00	19.19	A
ATOM	502	N	LEU	168	-8.720	6.735	17.209	1.00	17.06	A
ATOM	503	H	LEU	168	-8.311	6.890	18.120	1.00	15.00	A
ATOM	504	CA	LEU	168	-7.925	6.625	15.992	1.00	16.60	A
ATOM	505	CB	LEU	168	-6.600	7.343	16.289	1.00	21.87	A
ATOM	506	CG	LEU	168	-6.247	8.745	15.716	1.00	22.69	A
ATOM	507	CD1	LEU	168	-5.119	9.410	16.539	1.00	21.20	A
ATOM	508	CD2	LEU	168	-7.436	9.617	15.361	1.00	18.38	A
ATOM	509	C	LEU	168	-7.686	5.136	15.604	1.00	14.84	A
ATOM	510	O	LEU	168	-7.282	4.278	16.392	1.00	15.89	A
ATOM	511	N	TYR	169	-7.943	4.873	14.300	1.00	10.57	A
ATOM	512	H	TYR	169	-8.313	5.659	13.807	1.00	15.00	A
ATOM	513	CA	TYR	169	-7.683	3.572	13.656	1.00	5.27	A
ATOM	514	CB	TYR	169	-8.989	3.014	13.230	1.00	5.83	A
ATOM	515	CG	TYR	169	-9.857	2.620	14.423	1.00	6.94	A
ATOM	516	CD1	TYR	169	-10.524	3.598	15.168	1.00	7.40	A
ATOM	517	CE1	TYR	169	-11.390	3.193	16.218	1.00	7.77	A
ATOM	518	CD2	TYR	169	-10.016	1.255	14.744	1.00	8.89	A
ATOM	519	CE2	TYR	169	-10.850	0.841	15.804	1.00	9.40	A
ATOM	520	CZ	TYR	169	-11.563	1.827	16.534	1.00	10.39	A
ATOM	521	OH	TYR	169	-12.443	1.410	17.534	1.00	7.99	A
ATOM	522	HH	TYR	169	-13.009	2.117	17.800	1.00	15.00	A
ATOM	523	C	TYR	169	-6.810	3.642	12.390	1.00	6.72	A
ATOM	524	O	TYR	169	-6.917	4.498	11.557	1.00	9.12	A
ATOM	525	N	TYR	170	-5.899	2.722	12.228	1.00	9.53	A
ATOM	526	H	TYR	170	-5.806	2.081	12.986	1.00	15.00	A
ATOM	527	CA	TYR	170	-5.313	2.511	10.899	1.00	10.01	A
ATOM	528	CB	TYR	170	-3.967	1.797	11.044	1.00	7.46	A
ATOM	529	CG	TYR	170	-3.259	1.636	9.679	1.00	13.45	A
ATOM	530	CD1	TYR	170	-2.680	2.766	9.052	1.00	12.66	A
ATOM	531	CE1	TYR	170	-2.213	2.658	7.738	1.00	10.18	A
ATOM	532	CD2	TYR	170	-3.304	0.385	9.057	1.00	10.90	A
ATOM	533	CE2	TYR	170	-2.891	0.303	7.730	1.00	8.68	A
ATOM	534	CZ	TYR	170	-2.331	1.419	7.124	1.00	9.97	A
ATOM	535	OH	TYR	170	-1.774	1.286	5.859	1.00	17.50	A
ATOM	536	HH	TYR	170	-1.886	0.404	5.514	1.00	15.00	A
ATOM	537	C	TYR	170	-6.279	1.610	10.073	1.00	10.40	A
ATOM	538	O	TYR	170	-6.679	0.500	10.421	1.00	12.52	A
ATOM	539	N	ILE	171	-6.704	2.174	8.968	1.00	12.16	A

FIGURE 17J

ATOM	540	H	ILE	171	-6.475	3.135	8.808	1.00	15.00	A
ATOM	541	CA	ILE	171	-7.608	1.430	8.138	1.00	9.37	A
ATOM	542	CB	ILE	171	-9.070	1.990	8.317	1.00	11.21	A
ATOM	543	CG2	ILE	171	-9.326	3.501	8.677	1.00	17.27	A
ATOM	544	CG1	ILE	171	-10.046	1.564	7.214	1.00	13.33	A
ATOM	545	CD1	ILE	171	-10.647	0.250	7.619	1.00	17.53	A
ATOM	546	C	ILE	171	-7.074	1.234	6.694	1.00	8.34	A
ATOM	547	O	ILE	171	-6.453	2.088	6.082	1.00	6.96	A
ATOM	548	N	TYR	172	-7.286	0.005	6.216	1.00	11.07	A
ATOM	549	H	TYR	172	-7.809	-0.624	6.786	1.00	15.00	A
ATOM	550	CA	TYR	172	-6.708	-0.378	4.922	1.00	15.60	A
ATOM	551	CB	TYR	172	-5.332	-1.082	5.037	1.00	14.32	A
ATOM	552	CG	TYR	172	-5.389	-2.397	5.796	1.00	9.21	A
ATOM	553	CD1	TYR	172	-5.342	-2.402	7.216	1.00	12.52	A
ATOM	554	CE1	TYR	172	-5.607	-3.620	7.901	1.00	10.88	A
ATOM	555	CD2	TYR	172	-5.565	-3.586	5.050	1.00	12.66	A
ATOM	556	CE2	TYR	172	-5.829	-4.800	5.740	1.00	15.83	A
ATOM	557	CZ	TYR	172	-5.822	-4.808	7.164	1.00	11.94	A
ATOM	558	OH	TYR	172	-5.995	-6.002	7.820	1.00	12.17	A
ATOM	559	HH	TYR	172	-6.433	-5.843	8.657	1.00	15.00	A
ATOM	560	C	TYR	172	-7.605	-1.276	4.106	1.00	16.85	A
ATOM	561	O	TYR	172	-8.346	-2.057	4.692	1.00	14.06	A
ATOM	562	N	ALA	173	-7.448	-1.141	2.776	1.00	16.29	A
ATOM	563	H	ALA	173	-6.751	-0.490	2.503	1.00	15.00	A
ATOM	564	CA	ALA	173	-7.940	-2.152	1.836	1.00	15.11	A
ATOM	565	CB	ALA	173	-9.300	-1.725	1.292	1.00	12.08	A
ATOM	566	C	ALA	173	-7.007	-2.537	0.653	1.00	15.86	A
ATOM	567	O	ALA	173	-6.147	-1.806	0.191	1.00	14.20	A
ATOM	568	N	GLN	174	-7.244	-3.714	0.109	1.00	16.56	A
ATOM	569	H	GLN	174	-7.774	-4.389	0.620	1.00	15.00	A
ATOM	570	CA	GLN	174	-6.470	-4.119	-1.070	1.00	19.25	A
ATOM	571	CB	GLN	174	-5.582	-5.292	-0.832	1.00	21.99	A
ATOM	572	CG	GLN	174	-4.205	-4.727	-1.030	1.00	30.99	A
ATOM	573	CD	GLN	174	-3.174	-5.845	-0.979	1.00	34.25	A
ATOM	574	OE1	GLN	174	-2.308	-5.899	-0.105	1.00	32.91	A
ATOM	575	NE2	GLN	174	-3.268	-6.699	-2.014	1.00	31.50	A
ATOM	576	HE21	GLN	174	-2.668	-7.487	-1.970	1.00	15.00	A
ATOM	577	HE22	GLN	174	-3.973	-6.621	-2.714	1.00	15.00	A
ATOM	578	C	GLN	174	-7.413	-4.644	-2.114	1.00	19.20	A
ATOM	579	O	GLN	174	-8.285	-5.434	-1.880	1.00	20.03	A
ATOM	580	N	VAL	175	-7.291	-4.107	-3.301	1.00	19.28	A
ATOM	581	H	VAL	175	-6.594	-3.401	-3.400	1.00	15.00	A
ATOM	582	CA	VAL	175	-8.247	-4.500	-4.323	1.00	22.43	A
ATOM	583	CB	VAL	175	-9.319	-3.409	-4.644	1.00	21.41	A
ATOM	584	CG1	VAL	175	-10.146	-2.830	-3.495	1.00	20.17	A
ATOM	585	CG2	VAL	175	-10.268	-4.061	-5.639	1.00	22.88	A
ATOM	586	C	VAL	175	-7.508	-4.859	-5.615	1.00	24.56	A
ATOM	587	O	VAL	175	-6.928	-3.997	-6.301	1.00	23.28	A
ATOM	588	N	THR	176	-7.563	-6.180	-5.879	1.00	25.40	A
ATOM	589	H	THR	176	-7.994	-6.850	-5.250	1.00	15.00	A
ATOM	590	CA	THR	176	-7.086	-6.501	-7.222	1.00	24.46	A
ATOM	591	CB	THR	176	-5.844	-7.454	-7.256	1.00	24.78	A
ATOM	592	OG1	THR	176	-5.948	-8.650	-8.028	1.00	20.31	A
ATOM	593	HG1	THR	176	-5.250	-9.253	-7.796	1.00	15.00	A
ATOM	594	CG2	THR	176	-5.329	-7.711	-5.867	1.00	17.07	A
ATOM	595	C	THR	176	-8.178	-6.700	-8.272	1.00	25.44	A
ATOM	596	O	THR	176	-9.326	-7.043	-7.995	1.00	26.86	A
ATOM	597	N	PHE	177	-7.855	-6.341	-9.506	1.00	22.44	A
ATOM	598	H	PHE	177	-6.920	-6.083	-9.732	1.00	15.00	A
ATOM	599	CA	PHE	177	-8.939	-6.511	-10.479	1.00	22.70	A

FIGURE 17K

ATOM	600	CB	PHE	177	-9.746	-5.194	-10.599	1.00	20.90	A
ATOM	601	CG	PHE	177	-8.813	-4.034	-10.927	1.00	22.51	A
ATOM	602	CD1	PHE	177	-8.771	-3.546	-12.252	1.00	22.11	A
ATOM	603	CD2	PHE	177	-8.011	-3.422	-9.920	1.00	21.87	A
ATOM	604	CE1	PHE	177	-8.041	-2.367	-12.550	1.00	20.53	A
ATOM	605	CE2	PHE	177	-7.289	-2.247	-10.204	1.00	20.44	A
ATOM	606	CZ	PHE	177	-7.376	-1.713	-11.500	1.00	22.79	A
ATOM	607	C	PHE	177	-8.381	-6.949	-11.800	1.00	22.14	A
ATOM	608	O	PHE	177	-7.219	-6.695	-12.072	1.00	21.60	A
ATOM	609	N	CYS	178	-9.210	-7.555	-12.625	1.00	24.52	A
ATOM	610	H	CYS	178	-10.146	-7.797	-12.370	1.00	15.00	A
ATOM	611	CA	CYS	178	-8.599	-7.849	-13.942	1.00	29.77	A
ATOM	612	CB	CYS	178	-8.501	-9.365	-14.214	1.00	32.06	A
ATOM	613	SG	CYS	178	-7.685	-9.731	-15.792	1.00	35.17	A
ATOM	614	C	CYS	178	-9.323	-7.146	-15.088	1.00	28.41	A
ATOM	615	O	CYS	178	-10.534	-7.247	-15.185	1.00	27.54	A
ATOM	616	N	SER	179	-8.589	-6.393	-15.910	1.00	28.86	A
ATOM	617	H	SER	179	-7.608	-6.271	-15.754	1.00	15.00	A
ATOM	618	CA	SER	179	-9.374	-5.454	-16.704	1.00	29.01	A
ATOM	619	CB	SER	179	-9.379	-4.118	-16.020	1.00	30.82	A
ATOM	620	OG	SER	179	-10.615	-3.492	-16.319	1.00	39.79	A
ATOM	621	HG	SER	179	-10.725	-2.812	-15.667	1.00	15.00	A
ATOM	622	C	SER	179	-9.063	-5.196	-18.165	1.00	31.16	A
ATOM	623	O	SER	179	-7.931	-4.953	-18.537	1.00	28.58	A
ATOM	624	N	ASN	180	-10.083	-5.255	-19.042	1.00	35.32	A
ATOM	625	H	ASN	180	-10.966	-5.700	-18.834	1.00	15.00	A
ATOM	626	CA	ASN	180	-9.782	-4.725	-20.366	1.00	34.74	A
ATOM	627	CB	ASN	180	-10.205	-5.554	-21.589	1.00	37.96	A
ATOM	628	CG	ASN	180	-9.650	-4.980	-22.896	1.00	37.12	A
ATOM	629	OD1	ASN	180	-10.058	-3.947	-23.356	1.00	40.66	A
ATOM	630	ND2	ASN	180	-8.619	-5.536	-23.456	1.00	35.85	A
ATOM	631	HD21	ASN	180	-8.343	-6.475	-23.306	1.00	15.00	A
ATOM	632	HD22	ASN	180	-8.153	-4.891	-24.065	1.00	15.00	A
ATOM	633	C	ASN	180	-10.197	-3.331	-20.588	1.00	36.96	A
ATOM	634	O	ASN	180	-11.314	-2.894	-20.433	1.00	37.89	A
ATOM	635	N	ARG	181	-9.147	-2.699	-21.068	1.00	41.95	A
ATOM	636	H	ARG	181	-6.363	-3.318	-21.141	1.00	15.00	A
ATOM	637	CA	ARG	181	-8.997	-1.313	-21.489	1.00	44.24	A
ATOM	638	CB	ARG	181	-7.563	-1.279	-22.026	1.00	43.43	A
ATOM	639	CG	ARG	181	-6.348	-1.638	-21.101	1.00	45.11	A
ATOM	640	CD	ARG	181	-6.235	-2.853	-20.134	1.00	40.68	A
ATOM	641	NE	ARG	181	-5.064	-2.772	-19.271	1.00	46.11	A
ATOM	642	HE	ARG	181	-4.991	-2.058	-18.578	1.00	15.00	A
ATOM	643	CZ	ARG	181	-4.024	-3.611	-19.432	1.00	49.77	A
ATOM	644	NH1	ARG	181	-2.886	-3.414	-18.790	1.00	54.33	A
ATOM	645	HH11	ARG	181	-2.113	-4.032	-18.918	1.00	15.00	A
ATOM	646	HH12	ARG	181	-2.807	-2.642	-18.161	1.00	15.00	A
ATOM	647	NH2	ARG	181	-4.085	-4.641	-20.247	1.00	54.26	A
ATOM	648	HH21	ARG	181	-3.286	-5.230	-20.354	1.00	15.00	A
ATOM	649	HH22	ARG	181	-4.918	-4.833	-20.761	1.00	15.00	A
ATOM	650	C	ARG	181	-10.049	-0.866	-22.499	1.00	47.10	A
ATOM	651	O	ARG	181	-10.979	-0.112	-22.227	1.00	49.20	A
ATOM	652	N	GLU	182	-9.895	-1.447	-23.690	1.00	49.64	A
ATOM	653	H	GLU	182	-9.201	-2.166	-23.775	1.00	15.00	A
ATOM	654	CA	GLU	182	-10.976	-1.385	-24.676	1.00	52.41	A
ATOM	655	CB	GLU	182	-10.437	-2.020	-25.970	1.00	56.93	A
ATOM	656	CG	GLU	182	-10.932	-1.418	-27.295	1.00	66.05	A
ATOM	657	CD	GLU	182	-10.758	0.116	-27.327	1.00	70.54	A
ATOM	658	OE1	GLU	182	-9.613	0.586	-27.442	1.00	72.98	A
ATOM	659	OE2	GLU	182	-11.778	0.830	-27.244	1.00	72.46	A

FIGURE 17L

ATOM	660	C	GLU	182	-12.388	-1.934	-24.304	1.00	53.00	A
ATOM	661	O	GLU	182	-13.379	-1.492	-24.862	1.00	54.27	A
ATOM	662	N	ALA	183	-12.505	-2.877	-23.335	1.00	52.34	A
ATOM	663	H	ALA	183	-11.676	-3.173	-22.865	1.00	15.00	A
ATOM	664	CA	ALA	183	-13.867	-3.258	-22.899	1.00	50.19	A
ATOM	665	CB	ALA	183	-13.855	-4.721	-22.447	1.00	45.02	A
ATOM	666	C	ALA	183	-14.562	-2.321	-21.867	1.00	50.66	A
ATOM	667	O	ALA	183	-15.712	-1.945	-21.990	1.00	47.77	A
ATOM	668	N	SER	184	-13.773	-1.888	-20.878	1.00	52.95	A
ATOM	669	H	SER	184	-12.826	-2.172	-20.991	1.00	15.00	A
ATOM	670	CA	SER	184	-14.228	-1.043	-19.729	1.00	56.78	A
ATOM	671	CB	SER	184	-13.384	-1.397	-18.481	1.00	53.58	A
ATOM	672	OG	SER	184	-13.975	-2.448	-17.721	1.00	47.46	A
ATOM	673	HG	SER	184	-13.291	-3.019	-17.388	1.00	15.00	A
ATOM	674	C	SER	184	-14.183	0.517	-19.880	1.00	59.95	A
ATOM	675	O	SER	184	-13.913	1.297	-18.964	1.00	65.25	A
ATOM	676	N	SER	185	-14.324	0.995	-21.131	1.00	60.08	A
ATOM	677	H	SER	185	-14.623	0.345	-21.831	1.00	15.00	A
ATOM	678	CA	SER	185	-13.825	2.375	-21.391	1.00	60.12	A
ATOM	679	CB	SER	185	-13.522	2.640	-22.869	1.00	60.49	A
ATOM	680	OG	SER	185	-12.243	2.098	-23.242	1.00	59.80	A
ATOM	681	HG	SER	185	-12.158	1.234	-22.833	1.00	15.00	A
ATOM	682	C	SER	185	-14.580	3.589	-20.885	1.00	59.59	A
ATOM	683	O	SER	185	-15.437	4.159	-21.543	1.00	60.08	A
ATOM	684	N	GLN	186	-14.200	3.990	-19.670	1.00	57.71	A
ATOM	685	H	GLN	186	-13.601	3.376	-19.153	1.00	15.00	A
ATOM	686	CA	GLN	186	-15.121	4.936	-18.993	1.00	57.00	A
ATOM	687	CB	GLN	186	-16.094	4.062	-18.175	1.00	58.66	A
ATOM	688	CG	GLN	186	-15.355	3.354	-17.050	1.00	59.69	A
ATOM	689	CD	GLN	186	-16.369	2.789	-16.088	1.00	59.92	A
ATOM	690	OE1	GLN	186	-17.270	3.513	-15.687	1.00	59.81	A
ATOM	691	NE2	GLN	186	-16.249	1.503	-15.787	1.00	59.63	A
ATOM	692	HE21	GLN	186	-15.492	0.948	-16.113	1.00	15.00	A
ATOM	693	HE22	GLN	186	-16.950	1.119	-15.168	1.00	15.00	A
ATOM	694	C	GLN	186	-14.758	6.290	-18.221	1.00	54.36	A
ATOM	695	O	GLN	186	-15.596	7.198	-18.298	1.00	53.98	A
ATOM	696	N	ALA	187	-13.566	6.424	-17.511	1.00	50.35	A
ATOM	697	H	ALA	187	-13.476	7.274	-16.970	1.00	15.00	A
ATOM	698	CA	ALA	187	-12.388	5.599	-17.832	1.00	43.26	A
ATOM	699	CB	ALA	187	-11.546	6.284	-18.918	1.00	38.95	A
ATOM	700	C	ALA	187	-11.456	4.882	-16.849	1.00	40.48	A
ATOM	701	O	ALA	187	-10.887	3.875	-17.295	1.00	43.24	A
ATOM	702	N	PRO	188	-11.210	5.383	-15.594	1.00	38.66	A
ATOM	703	CD	PRO	188	-11.543	6.687	-15.000	1.00	38.15	A
ATOM	704	CA	PRO	188	-10.220	4.665	-14.751	1.00	35.94	A
ATOM	705	CB	PRO	188	-9.395	5.813	-14.150	1.00	33.99	A
ATOM	706	CG	PRO	188	-10.377	7.000	-14.036	1.00	32.69	A
ATOM	707	C	PRO	188	-10.840	3.783	-13.683	1.00	33.66	A
ATOM	708	O	PRO	188	-11.885	4.062	-13.140	1.00	33.41	A
ATOM	709	N	PHE	189	-10.147	2.695	-13.346	1.00	28.66	A
ATOM	710	H	PHE	189	-9.260	2.508	-13.748	1.00	15.00	A
ATOM	711	CA	PHE	189	-10.721	2.013	-12.171	1.00	26.71	A
ATOM	712	CB	PHE	189	-10.122	0.601	-12.034	1.00	26.21	A
ATOM	713	CG	PHE	189	-10.671	-0.189	-10.849	1.00	22.92	A
ATOM	714	CD1	PHE	189	-10.126	0.005	-9.566	1.00	17.72	A
ATOM	715	CD2	PHE	189	-11.687	-1.165	-11.064	1.00	21.88	A
ATOM	716	CE1	PHE	189	-10.590	-0.815	-8.522	1.00	19.12	A
ATOM	717	CE2	PHE	189	-12.124	-1.995	-10.011	1.00	21.13	A
ATOM	718	CZ	PHE	189	-11.571	-1.806	-8.736	1.00	18.44	A
ATOM	719	C	PHE	189	-10.445	2.815	-10.909	1.00	27.14	A

FIGURE 17M

ATOM	720	O	PHE	189	-9.308	3.244	-10.706	1.00	25.72	A
ATOM	721	N	ILE	190	-11.468	2.964	-10.071	1.00	24.71	A
ATOM	722	H	ILE	190	-12.408	2.786	-10.389	1.00	15.00	A
ATOM	723	CA	ILE	190	-11.193	3.626	-8.788	1.00	24.03	A
ATOM	724	CB	ILE	190	-11.316	5.242	-8.743	1.00	25.86	A
ATOM	725	CG2	ILE	190	-11.892	5.979	-9.997	1.00	19.67	A
ATOM	726	CG1	ILE	190	-11.801	5.888	-7.424	1.00	22.54	A
ATOM	727	CD1	ILE	190	-12.819	7.012	-7.645	1.00	28.56	A
ATOM	728	C	ILE	190	-11.844	2.812	-7.656	1.00	21.97	A
ATOM	729	O	ILE	190	-12.891	2.197	-7.801	1.00	16.30	A
ATOM	730	N	ALA	191	-11.026	2.700	-6.590	1.00	17.21	A
ATOM	731	H	ALA	191	-10.124	3.124	-6.662	1.00	15.00	A
ATOM	732	CA	ALA	191	-11.501	2.195	-5.321	1.00	15.20	A
ATOM	733	CB	ALA	191	-10.730	0.928	-4.968	1.00	14.79	A
ATOM	734	C	ALA	191	-11.439	3.230	-4.206	1.00	17.11	A
ATOM	735	O	ALA	191	-10.467	3.961	-4.052	1.00	14.04	A
ATOM	736	N	SER	192	-12.511	3.245	-3.433	1.00	14.72	A
ATOM	737	H	SER	192	-13.277	2.694	-3.804	1.00	15.00	A
ATOM	738	CA	SER	192	-12.725	4.289	-2.423	1.00	16.69	A
ATOM	739	CB	SER	192	-13.931	5.144	-2.803	1.00	14.83	A
ATOM	740	OG	SER	192	-13.556	5.828	-3.994	1.00	21.23	A
ATOM	741	HG	SER	192	-14.367	5.966	-4.520	1.00	15.00	A
ATOM	742	C	SER	192	-12.980	3.682	-1.069	1.00	17.77	A
ATOM	743	O	SER	192	-13.753	2.738	-0.947	1.00	20.76	A
ATOM	744	N	LEU	193	-12.285	4.209	-0.038	1.00	15.56	A
ATOM	745	H	LEU	193	-11.681	4.959	-0.280	1.00	15.00	A
ATOM	746	CA	LEU	193	-12.510	3.761	1.366	1.00	13.27	A
ATOM	747	CB	LEU	193	-11.195	3.825	2.217	1.00	12.74	A
ATOM	748	CG	LEU	193	-11.051	3.141	3.604	1.00	14.37	A
ATOM	749	CD1	LEU	193	-12.272	2 354	4.116	1.00	14.67	A
ATOM	750	CD2	LEU	193	-10.274	3 986	4.622	1.00	12.64	A
ATOM	751	C	LEU	193	-13.497	4.748	1.911	1.00	11.22	A
ATOM	752	O	LEU	193	-13.188	5.912	1.903	1.00	12.22	A
ATOM	753	N	CYS	194	-14 652	4.326	2.310	1.00	13.66	A
ATOM	754	H	CYS	194	-14.828	3.347	2.276	1.00	15.00	A
ATOM	755	CA	CYS	194	-15 595	5.360	2.713	1.00	14.84	A
ATOM	756	CB	CYS	194	-16 915	5.409	1.918	1.00	17.58	A
ATOM	757	SG	CYS	194	-16 623	5.417	0.165	1.00	16.33	A
ATOM	758	C	CYS	194	-16.046	5.163	4.137	1.00	12.81	A
ATOM	759	O	CYS	194	-15 983	4.072	4.655	1.00	10.34	A
ATOM	760	N	LEU	195	-16 557	6 254	4.697	1.00	14.32	A
ATOM	761	H	LEU	195	-16 541	7 088	4.154	1.00	15.00	A
ATOM	762	CA	LEU	195	-17 039	6 291	6.076	1.00	14.89	A
ATOM	763	CB	LEU	195	-16.295	7 372	6.789	1.00	15.56	A
ATOM	764	CG	LEU	195	-16.571	7.680	8.242	1.00	15.56	A
ATOM	765	CD1	LEU	195	-15.932	8.967	8.762	1.00	13.72	A
ATOM	766	CD2	LEU	195	-16.463	6.448	9.154	1.00	17.25	A
ATOM	767	C	LEU	195	-18.546	6.544	6.209	1.00	13.54	A
ATOM	768	O	LEU	195	-19.038	7.521	5.705	1.00	14.56	A
ATOM	769	N	LYS	196	-19.238	5.667	6.905	1.00	16.36	A
ATOM	770	H	LYS	196	-18.719	4.875	7.197	1.00	15.00	A
ATOM	771	CA	LYS	196	-20.577	5.972	7.405	1.00	21.01	A
ATOM	772	CB	LYS	196	-21.475	4.726	7.146	1.00	22.66	A
ATOM	773	CG	LYS	196	-22.953	4.839	7.590	1.00	31.25	A
ATOM	774	CD	LYS	196	-23.354	4.915	9.104	1.00	40.25	A
ATOM	775	CE	LYS	196	-23.189	3.694	10.060	1.00	43.56	A
ATOM	776	NZ	LYS	196	-23.004	4.158	11.453	1.00	44.46	A
ATOM	777	HZ1	LYS	196	-22.182	4.799	11.467	1.00	15.00	A
ATOM	778	HZ2	LYS	196	-23.847	4.665	11.778	1.00	15.00	A
ATOM	779	HZ3	LYS	196	-22.807	3.334	12.066	1.00	15.00	A

FIGURE 17N

ATOM	780	C	LYS	196	-20.478	6.290	8.899	1.00	19.25	A
ATOM	781	O	LYS	196	-20.194	5.434	9.714	1.00	18.35	A
ATOM	782	N	SER	197	-20.664	7.534	9.272	1.00	21.63	A
ATOM	783	H	SER	197	-20.891	8.247	8.615	1.00	15.00	A
ATOM	784	CA	SER	197	-20.752	7.701	10.729	1.00	24.87	A
ATOM	785	CB	SER	197	-19.898	8.878	11.207	1.00	25.61	A
ATOM	786	OG	SER	197	-19.563	8.687	12.588	1.00	32.22	A
ATOM	787	HG	SER	197	-18.795	8.110	12.611	1.00	15.00	A
ATOM	788	C	SER	197	-22.216	7.810	11.218	1.00	26.33	A
ATOM	789	O	SER	197	-23.078	8.303	10.497	1.00	26.57	A
ATOM	790	N	PRO	198	-22.534	7.274	12.407	1.00	26.77	A
ATOM	791	CD	PRO	198	-21.649	6.526	13.301	1.00	32.92	A
ATOM	792	CA	PRO	198	-23.919	7.381	12.913	1.00	28.73	A
ATOM	793	CB	PRO	198	-23.784	6.789	14.318	1.00	32.89	A
ATOM	794	CG	PRO	198	-22.289	6.726	14.659	1.00	33.55	A
ATOM	795	C	PRO	198	-24.591	8.789	12.847	1.00	26.60	A
ATOM	796	O	PRO	198	-24.035	9.817	13.242	1.00	20.20	A
ATOM	797	N	GLY	199	-25.729	8.773	12.119	1.00	25.75	A
ATOM	798	H	GLY	199	-26.170	7.857	12.057	1.00	15.00	A
ATOM	799	CA	GLY	199	-26.486	10.003	11.790	1.00	26.91	A
ATOM	800	C	GLY	199	-25.821	10.971	10.816	1.00	28.98	A
ATOM	801	O	GLY	199	-26.084	12.151	10.797	1.00	31.05	A
ATOM	802	N	ARG	200	-24.898	10.464	10.001	1.00	30.15	A
ATOM	803	H	ARG	200	-24.629	9.519	10.165	1.00	15.00	A
ATOM	804	CA	ARG	200	-24.140	11.384	9.166	1.00	28.98	A
ATOM	805	CB	ARG	200	-22.749	11.590	9.783	1.00	33.16	A
ATOM	806	CG	ARG	200	-22.739	12.290	11.162	1.00	38.34	A
ATOM	807	CD	ARG	200	-21.327	12.530	11.705	1.00	42.14	A
ATOM	808	NE	ARG	200	-21.292	12.875	13.131	1.00	43.64	A
ATOM	809	HE	ARG	200	-21.327	13.831	13.424	1.00	15.00	A
ATOM	810	CZ	ARG	200	-21.138	11.896	14.051	1.00	46.40	A
ATOM	811	NH1	ARG	200	-21.219	10.603	13.733	1.00	46.31	A
ATOM	812	HH11	ARG	200	-21.104	9.910	14.445	1.00	15.00	A
ATOM	813	HH12	ARG	200	-21.394	10.320	12.789	1.00	15.00	A
ATOM	814	NH2	ARG	200	-20.901	12.226	15.311	1.00	46.65	A
ATOM	815	HH21	ARG	200	-20.847	13.193	15.566	1.00	15.00	A
ATOM	816	HH22	ARG	200	-20.785	11.510	16.002	1.00	15.00	A
ATOM	817	C	ARG	200	-24.084	10.967	7.710	1.00	27.77	A
ATOM	818	O	ARG	200	-24.264	9.791	7.449	1.00	28.21	A
ATOM	819	N	PHE	201	-23.853	11.926	6.792	1.00	30.83	A
ATOM	820	H	PHE	201	-23.513	12.821	7.126	1.00	15.00	A
ATOM	821	CA	PHE	201	-24.016	11.708	5.339	1.00	34.17	A
ATOM	822	CB	PHE	201	-23.851	12.996	4.572	1.00	31.58	A
ATOM	823	CG	PHE	201	-25.154	13.730	4.614	1.00	34.85	A
ATOM	824	CD1	PHE	201	-25.174	15.062	5.081	1.00	37.56	A
ATOM	825	CD2	PHE	201	-26.335	13.081	4.190	1.00	37.89	A
ATOM	826	CE1	PHE	201	-26.397	15.749	5.182	1.00	36.91	A
ATOM	827	CE2	PHE	201	-27.566	13.762	4.280	1.00	38.98	A
ATOM	828	CZ	PHE	201	-27.572	15.065	4.815	1.00	37.61	A
ATOM	829	C	PHE	201	-23.277	10.605	4.545	1.00	39.40	A
ATOM	830	O	PHE	201	-23.853	10.034	3.604	1.00	45.71	A
ATOM	831	N	GLU	202	-22.031	10.316	5.034	1.00	35.75	A
ATOM	832	H	GLU	202	-21.878	10.753	5.925	1.00	15.00	A
ATOM	833	CA	GLU	202	-20.964	9.564	4.318	1.00	34.52	A
ATOM	834	CB	GLU	202	-21.295	8.540	3.234	1.00	33.66	A
ATOM	835	CG	GLU	202	-21.924	7.245	3.713	1.00	40.61	A
ATOM	836	CD	GLU	202	-22.647	6.505	2.561	1.00	46.12	A
ATOM	837	OE1	GLU	202	-23.461	5.613	2.886	1.00	46.89	A
ATOM	838	OE2	GLU	202	-22.417	6.814	1.370	1.00	45.63	A
ATOM	839	C	GLU	202	-19.924	10.450	3.717	1.00	29.99	A

FIGURE 17O

ATOM	840	O	GLU	202	-20.137	11.567	3.300	1.00	[illegible]	A
ATOM	841	N	ARG	203	-19.728	9.897	3.856	1.00	26.88	A
ATOM	842	H	ARG	203	-18.690	8.998	4.285	1.00	15.00	A
ATOM	843	CA	ARG	203	-17.539	10.603	3.358	1.00	21.88	A
ATOM	844	CB	ARG	203	-16.819	11.410	4.457	1.00	[illegible]	A
ATOM	845	CG	ARG	203	-17.681	12.187	5.467	1.00	37.32	A
ATOM	846	CD	ARG	203	-16.894	13.213	6.339	1.00	48.09	A
ATOM	847	NE	ARG	203	-15.911	12.667	7.308	1.00	56.90	A
ATOM	848	HE	ARG	203	-16.240	12.433	8.223	1.00	15.00	A
ATOM	849	CZ	ARG	203	-14.572	12.475	7.001	1.00	66.77	A
ATOM	850	NH1	ARG	203	-13.702	12.002	7.911	1.00	68.44	A
ATOM	851	HH11	ARG	203	-12.745	11.829	7.666	1.00	15.00	A
ATOM	852	HH12	ARG	203	-14.016	11.822	8.845	1.00	15.00	A
ATOM	853	NH2	ARG	203	-14.084	12.716	5.766	1.00	67.68	A
ATOM	854	HH21	ARG	203	-14.670	13.108	5.060	1.00	15.00	A
ATOM	855	HH22	ARG	203	-13.143	12.499	5.544	1.00	15.00	A
ATOM	856	C	ARG	203	-16.517	9.633	2.678	1.00	17.71	A
ATOM	857	O	ARG	203	-16.375	8.418	2.931	1.00	7.69	A
ATOM	858	N	ILE	204	-15.789	10.253	1.791	1.00	14.42	A
ATOM	859	H	ILE	204	-15.915	11.228	1.561	1.00	15.00	A
ATOM	860	CA	ILE	204	-14.662	9.482	1.353	1.00	18.32	A
ATOM	861	CB	ILE	204	-14.520	9.392	-0.231	1.00	24.52	A
ATOM	862	CG2	ILE	204	-15.820	9.529	-1.069	1.00	21.85	A
ATOM	863	CG1	ILE	204	-13.439	10.195	-0.949	1.00	26.35	A
ATOM	864	CD1	ILE	204	-13.992	11.231	-1.961	1.00	36.33	A
ATOM	865	C	ILE	204	-13.387	9.819	2.153	1.00	16.58	A
ATOM	866	O	ILE	204	-13.070	10.956	2.457	1.00	18.63	A
ATOM	867	N	LEU	205	-12.718	8.725	2.571	1.00	13.32	A
ATOM	868	H	LEU	205	-13.142	7.853	2.321	1.00	15.00	A
ATOM	869	CA	LEU	205	-11.467	8.829	3.322	1.00	10.01	A
ATOM	870	CB	LEU	205	-11.440	7.688	4.382	1.00	6.66	A
ATOM	871	CG	LEU	205	-12.571	7.727	5.441	1.00	7.99	A
ATOM	872	CD1	LEU	205	-12.722	9.088	6.089	1.00	8.78	A
ATOM	873	CD2	LEU	205	-12.419	6.720	6.582	1.00	8.08	A
ATOM	874	C	LEU	205	-10.268	8.811	2.377	1.00	9.75	A
ATOM	875	O	LEU	205	-9.416	9.655	2.320	1.00	10.25	A
ATOM	876	N	LEU	206	-10.252	7.769	1.562	1.00	10.28	A
ATOM	877	H	LEU	206	-10.991	7.119	1.684	1.00	15.00	A
ATOM	878	CA	LEU	206	-9.166	7.555	0.610	1.00	10.02	A
ATOM	879	CB	LEU	206	-8.249	6.384	0.990	1.00	11.94	A
ATOM	880	CG	LEU	206	-7.001	6.527	1.859	1.00	14.40	A
ATOM	881	CD1	LEU	206	-7.094	5.595	3.074	1.00	14.49	A
ATOM	882	CD2	LEU	206	-6.531	7.958	2.151	1.00	8.78	A
ATOM	883	C	LEU	206	9.756	7.071	-0.697	1.00	11.91	A
ATOM	884	O	LEU	206	-10.792	6.406	-0.778	1.00	10.67	A
ATOM	885	N	ARG	207	-9.005	7.428	-1.720	1.00	8.06	A
ATOM	886	H	ARG	207	-8.196	7.992	-1.553	1.00	15.00	A
ATOM	887	CA	ARG	207	-9.309	6.823	-2.992	1.00	10.45	A
ATOM	888	CB	ARG	207	-9.974	7.790	-3.904	1.00	8.71	A
ATOM	889	CG	ARG	207	-11.258	8.270	-3.357	1.00	15.68	A
ATOM	890	CD	ARG	207	-11.652	9.459	-4.163	1.00	22.25	A
ATOM	891	NE	ARG	207	-12.670	9.192	-5.171	1.00	29.59	A
ATOM	892	HE	ARG	207	-13.115	8.300	-5.249	1.00	15.00	A
ATOM	893	CZ	ARG	207	-13.063	10.272	-5.919	1.00	40.09	A
ATOM	894	NH1	ARG	207	-12.482	11.498	-5.813	1.00	36.32	A
ATOM	895	HH11	ARG	207	-12.813	12.246	-6.391	1.00	15.00	A
ATOM	896	HH12	ARG	207	-11.737	11.651	-5.165	1.00	15.00	A
ATOM	897	NH2	ARG	207	-14.067	10.111	-6.773	1.00	40.86	A
ATOM	898	HH21	ARG	207	-14.392	10.877	-7.329	1.00	15.00	A
ATOM	899	HH22	ARG	207	-14.498	9.207	-6.853	1.00	15.00	A

FIGURE 17P

ATOM	900	C	ARG	207	-8.044	6.456	-3.741	1.00	12.59	A
ATOM	901	O	ARG	207	-7.053	7.150	-3.787	1.00	15.58	A
ATOM	902	N	ALA	208	-8.096	5.358	-4.465	1.00	17.06	A
ATOM	903	H	ALA	208	-8.679	4.758	-4.355	1.00	15.00	A
ATOM	904	CA	ALA	208	-7.025	5.128	-5.465	1.00	17.00	A
ATOM	905	CB	ALA	208	-6.052	4.020	-5.072	1.00	14.69	A
ATOM	906	C	ALA	208	-7.544	4.830	-6.854	1.00	20.46	A
ATOM	907	O	ALA	208	-8.438	4.020	-7.057	1.00	21.89	A
ATOM	908	N	ALA	209	-6.986	5.586	-7.808	1.00	26.22	A
ATOM	909	H	ALA	209	-6.280	6.235	-7.533	1.00	15.00	A
ATOM	910	CA	ALA	209	-7.253	5.208	-9.196	1.00	28.06	A
ATOM	911	CB	ALA	209	-7.702	6.380	-10.069	1.00	27.10	A
ATOM	912	C	ALA	209	-6.075	4.461	-9.832	1.00	32.54	A
ATOM	913	O	ALA	209	-4.895	4.726	-9.593	1.00	33.00	A
ATOM	914	N	ASN	210	-6.502	3.491	-10.634	1.00	32.11	A
ATOM	915	H	ASN	210	-7.466	3.249	-10.531	1.00	15.00	A
ATOM	916	CA	ASN	210	-5.674	2.893	-11.662	1.00	36.00	A
ATOM	917	CB	ASN	210	-5.366	1.446	-11.355	1.00	39.53	A
ATOM	918	CG	ASN	210	-4.463	1.366	-10.154	1.00	42.59	A
ATOM	919	OD1	ASN	210	-4.285	2.273	-9.342	1.00	39.26	A
ATOM	920	ND2	ASN	210	-3.951	0.165	-10.055	1.00	41.77	A
ATOM	921	HD21	ASN	210	-3.990	-0.479	-10.817	1.00	15.00	A
ATOM	922	HD22	ASN	210	-3.364	-0.081	-9.279	1.00	15.00	A
ATOM	923	C	ASN	210	-6.299	2.931	-13.043	1.00	36.95	A
ATOM	924	O	ASN	210	-7.492	2.752	-13.259	1.00	36.93	A
ATOM	925	N	THR	211	-5.447	3.168	-14.013	1.00	37.83	A
ATOM	926	H	THR	211	-4.484	3.377	-13.821	1.00	15.00	A
ATOM	927	CA	THR	211	-6.119	3.224	-15.314	1.00	41.27	A
ATOM	928	CB	THR	211	-5.325	4.158	-16.268	1.00	44.53	A
ATOM	929	OG1	THR	211	-6.076	4.506	-17.438	1.00	49.34	A
ATOM	930	HG1	THR	211	-6.032	5.493	-17.508	1.00	15.00	A
ATOM	931	CG2	THR	211	-3.926	3.604	-16.581	1.00	46.08	A
ATOM	932	C	THR	211	-6.434	1.833	-15.878	1.00	39.17	A
ATOM	933	O	THR	211	-5.822	0.863	-15.475	1.00	36.48	A
ATOM	934	N	HIS	212	-7.416	1.718	-16.789	1.00	37.14	A
ATOM	935	H	HIS	212	-8.106	2.438	-16.878	1.00	15.00	A
ATOM	936	CA	HIS	212	-7.294	0.454	-17.529	1.00	33.23	A
ATOM	937	CB	HIS	212	-8.680	-0.012	-18.082	1.00	27.73	A
ATOM	938	CG	HIS	212	-9.856	0.060	-17.111	1.00	24.58	A
ATOM	939	ND1	HIS	212	-10.862	0.967	-17.161	1.00	24.59	A
ATOM	940	HD1	HIS	212	-11.000	1.702	-17.794	1.00	15.00	A
ATOM	941	CD2	HIS	212	-10.049	-0.723	-15.985	1.00	20.65	A
ATOM	942	NE2	HIS	212	-11.154	-0.265	-15.383	1.00	24.01	A
ATOM	943	CE1	HIS	212	-11.665	0.780	-16.092	1.00	17.59	A
ATOM	944	C	HIS	212	-6.257	0.633	-18.683	1.00	38.31	A
ATOM	945	O	HIS	212	-5.363	-0.132	-18.923	1.00	33.92	A
ATOM	946	N	SER	213	-6.444	1.737	-19.443	1.00	46.63	A
ATOM	947	H	SER	213	-7.156	2.323	-19.055	1.00	15.00	A
ATOM	948	CA	SER	213	-5.705	2.177	-20.675	1.00	53.91	A
ATOM	949	CB	SER	213	-4.272	2.704	-20.400	1.00	52.61	A
ATOM	950	OG	SER	213	-3.266	1.697	-20.547	1.00	53.97	A
ATOM	951	HG	SER	213	-3.363	1.064	-19.823	1.00	15.00	A
ATOM	952	C	SER	213	-5.844	1.508	-22.097	1.00	60.03	A
ATOM	953	O	SER	213	-5.005	0.811	-22.682	1.00	61.19	A
ATOM	954	N	SER	214	-7.043	1.803	-22.686	1.00	64.96	A
ATOM	955	H	SER	214	-7.705	2.322	-22.146	1.00	15.00	A
ATOM	956	CA	SER	214	-7.463	1.456	-24.094	1.00	69.62	A
ATOM	957	CB	SER	214	8.727	2.218	-24.495	1.00	67.82	A
ATOM	958	OG	SER	214	-9.563	2.257	-23.336	1.00	67.64	A
ATOM	959	HG	SER	214	-10.468	2.398	-23.623	1.00	15.00	A

FIGURE 17Q

ATOM	960	C	SER	214	-6.518	1.587	-25.300	1.00	72.08	A
ATOM	961	O	SER	214	-6.102	2.653	-25.686	1.00	73.45	A
ATOM	962	N	ALA	215	-6.175	0.409	-25.899	1.00	73.38	A
ATOM	963	H	ALA	215	-5.456	0.596	-26.565	1.00	15.00	A
ATOM	964	CA	ALA	215	-6.858	-0.915	-25.753	1.00	72.62	A
ATOM	965	CB	ALA	215	-7.199	-1.505	-27.138	1.00	73.08	A
ATOM	966	C	ALA	215	-6.331	-2.148	-24.983	1.00	72.11	A
ATOM	967	O	ALA	215	-7.020	-3.161	-25.069	1.00	72.74	A
ATOM	968	N	LYS	216	-5.153	-2.076	-24.282	1.00	70.17	A
ATOM	969	H	LYS	216	-4.747	-1.165	-24.199	1.00	15.00	A
ATOM	970	CA	LYS	216	-4.482	-3.256	-23.626	1.00	67.38	A
ATOM	971	CB	LYS	216	-3.458	-2.691	-22.648	1.00	65.30	A
ATOM	972	CG	LYS	216	-2.217	-2.107	-23.321	1.00	66.86	A
ATOM	973	CD	LYS	216	-1.419	-3.149	-24.134	1.00	68.81	A
ATOM	974	CE	LYS	216	-0.082	-2.674	-24.740	1.00	67.51	A
ATOM	975	NZ	LYS	216	0.483	-3.722	-25.598	1.00	67.80	A
ATOM	976	HZ1	LYS	216	0.620	-4.590	-25.041	1.00	15.00	A
ATOM	977	HZ2	LYS	216	-0.168	-3.914	-26.385	1.00	15.00	A
ATOM	978	HZ3	LYS	216	1.401	-3.406	-25.973	1.00	15.00	A
ATOM	979	C	LYS	216	-5.321	-4.441	-22.993	1.00	66.99	A
ATOM	980	O	LYS	216	-6.462	-4.266	-22.575	1.00	69.90	A
ATOM	981	N	PRO	217	-4.835	-5.724	-22.952	1.00	65.06	A
ATOM	982	CD	PRO	217	-3.525	-6.262	-23.308	1.00	67.91	A
ATOM	983	CA	PRO	217	-5.792	-6.827	-22.626	1.00	62.80	A
ATOM	984	CB	PRO	217	-5.285	-8.004	-23.464	1.00	64.33	A
ATOM	985	CG	PRO	217	-3.755	-7.799	-23.338	1.00	69.63	A
ATOM	986	C	PRO	217	-5.837	-7.237	-21.150	1.00	59.77	A
ATOM	987	O	PRO	217	-4.747	-7.318	-20.589	1.00	58.81	A
ATOM	988	N	CYS	218	-7.115	-7.516	-20.627	1.00	55.45	A
ATOM	989	H	CYS	218	-7.874	-7.287	-21.233	1.00	15.00	A
ATOM	990	CA	CYS	218	-7.433	-7.929	-19.210	1.00	46.55	A
ATOM	991	CB	CYS	218	-8.105	-9.289	-19.079	1.00	44.69	A
ATOM	992	SG	CYS	218	-8.855	-9.822	-17.460	1.00	43.11	A
ATOM	993	C	CYS	218	-6.265	-7.994	-18.263	1.00	43.24	A
ATOM	994	O	CYS	218	-5.720	-9.026	-17.959	1.00	44.68	A
ATOM	995	N	GLY	219	-5.853	-6.820	-17.876	1.00	40.28	A
ATOM	996	H	GLY	219	-6.328	-5.961	-18.059	1.00	15.00	A
ATOM	997	CA	GLY	219	-4.659	-6.828	-17.070	1.00	36.27	A
ATOM	998	C	GLY	219	-5.017	-7.080	-15.643	1.00	33.86	A
ATOM	999	O	GLY	219	-5.906	-6.452	-15.097	1.00	34.90	A
ATOM	1000	N	GLN	220	-4.313	-7.996	-15.023	1.00	33.15	A
ATOM	1001	H	GLN	220	-3.835	-8.684	-15.580	1.00	15.00	A
ATOM	1002	CA	GLN	220	-4.448	-7.929	-13.578	1.00	29.92	A
ATOM	1003	CB	GLN	220	-4.298	-9.282	-12.936	1.00	27.81	A
ATOM	1004	CG	GLN	220	-5.380	-9.340	-11.883	1.00	30.94	A
ATOM	1005	CD	GLN	220	-5.285	-10.631	-11.132	1.00	36.37	A
ATOM	1006	OE1	GLN	220	-4.216	-10.969	-10.661	1.00	38.47	A
ATOM	1007	NE2	GLN	220	-6.425	-11.296	-10.977	1.00	37.61	A
ATOM	1008	HE21	GLN	220	-6.295	-12.235	-10.667	1.00	15.00	A
ATOM	1009	HE22	GLN	220	-7.373	-11.036	-11.200	1.00	15.00	A
ATOM	1010	C	GLN	220	-3.666	-6.845	-12.859	1.00	27.48	A
ATOM	1011	O	GLN	220	-2.461	-6.694	-12.999	1.00	27.61	A
ATOM	1012	N	GLN	221	-4.438	-6.040	-12.110	1.00	25.10	A
ATOM	1013	H	GLN	221	-5.433	-6.174	-12.143	1.00	15.00	A
ATOM	1014	CA	GLN	221	-3.803	-4.929	-11.387	1.00	22.41	A
ATOM	1015	CB	GLN	221	-4.077	-3.528	-11.949	1.00	22.12	A
ATOM	1016	CG	GLN	221	-3.284	-3.029	-13.163	1.00	32.16	A
ATOM	1017	CD	GLN	221	-3.795	-1.637	-13.405	1.00	34.69	A
ATOM	1018	OE1	GLN	221	-3.746	-0.763	-12.558	1.00	42.12	A
ATOM	1019	NE2	GLN	221	-4.648	-1.507	-14.398	1.00	34.93	A

FIGURE 17R

ATOM	1020	HE21	GLN	221	-4.981	-2.187	-15.042	1.00	15.00	A
ATOM	1021	HE22	GLN	221	-4.844	-0.551	-14.575	1.00	15.00	A
ATOM	1022	C	GLN	221	-4.227	-4.913	-9.948	1.00	19.54	A
ATOM	1023	O	GLN	221	-5.300	-5.381	-9.611	1.00	19.46	A
ATOM	1024	N	SER	222	-3.374	-4.330	-9.123	1.00	18.12	A
ATOM	1025	H	SER	222	-2.442	-4.098	-9.441	1.00	15.00	A
ATOM	1026	CA	SER	222	-3.851	-4.120	-7.752	1.00	19.45	A
ATOM	1027	CB	SER	222	-3.104	-4.947	-6.691	1.00	19.99	A
ATOM	1028	OG	SER	222	-3.096	-6.339	-7.053	1.00	24.64	A
ATOM	1029	HG	SER	222	-2.651	-6.336	-7.904	1.00	15.00	A
ATOM	1030	C	SER	222	-3.731	-2.688	-7.330	1.00	24.09	A
ATOM	1031	O	SER	222	-2.992	-1.929	-7.944	1.00	29.41	A
ATOM	1032	N	ILE	223	-4.534	-2.386	-6.283	1.00	22.81	A
ATOM	1033	H	ILE	223	-5.172	-3.127	-6.074	1.00	15.00	A
ATOM	1034	CA	ILE	223	-4.567	-1.122	-5.530	1.00	21.06	A
ATOM	1035	CB	ILE	223	-5.970	-0.490	-5.852	1.00	19.87	A
ATOM	1036	CG2	ILE	223	-6.564	0.315	-4.673	1.00	16.59	A
ATOM	1037	CG1	ILE	223	-5.911	0.278	-7.188	1.00	15.22	A
ATOM	1038	CD1	ILE	223	-7.229	0.868	-7.709	1.00	20.54	A
ATOM	1039	C	ILE	223	-4.367	-1.446	-4.007	1.00	21.62	A
ATOM	1040	O	ILE	223	-5.098	-2.269	-3.444	1.00	19.58	A
ATOM	1041	N	HIS	224	-3.429	-0.767	-3.340	1.00	19.73	A
ATOM	1042	H	HIS	224	-2.794	-0.230	-3.899	1.00	15.00	A
ATOM	1043	CA	HIS	224	-3.497	-0.671	-1.858	1.00	16.45	A
ATOM	1044	CB	HIS	224	-2.164	-1.183	-1.227	1.00	18.74	A
ATOM	1045	CG	HIS	224	-2.182	-1.442	0.296	1.00	14.92	A
ATOM	1046	ND1	HIS	224	-2.479	-2.628	0.682	1.00	15.33	A
ATOM	1047	HD1	HIS	224	-2.667	-3.515	0.505	1.00	15.00	A
ATOM	1048	CD2	HIS	224	-1.964	-0.524	1.310	1.00	13.79	A
ATOM	1049	NE2	HIS	224	-2.137	-1.127	2.517	1.00	10.52	A
ATOM	1050	CE1	HIS	224	-2.458	-2.411	2.232	1.00	11.70	A
ATOM	1051	C	HIS	224	-3.914	0.699	-1.284	1.00	15.18	A
ATOM	1052	O	HIS	224	-3.338	1.732	-1.520	1.00	14.36	A
ATOM	1053	N	LEU	225	-4.970	0.673	-0.468	1.00	16.85	A
ATOM	1054	H	LEU	225	-5.317	-0.238	-0.252	1.00	15.00	A
ATOM	1055	CA	LEU	225	-5.395	1.885	0.256	1.00	15.55	A
ATOM	1056	CB	LEU	225	-6.927	2.082	0.208	1.00	17.15	A
ATOM	1057	CG	LEU	225	-7.495	2.456	-1.154	1.00	18.03	A
ATOM	1058	CD1	LEU	225	-6.792	3.659	-1.774	1.00	19.34	A
ATOM	1059	CD2	LEU	225	-8.994	2.659	-1.098	1.00	13.66	A
ATOM	1060	C	LEU	225	-5.074	1.758	1.739	1.00	14.77	A
ATOM	1061	O	LEU	225	-5.347	0.726	2.345	1.00	12.20	A
ATOM	1062	N	GLY	226	-4.544	2.829	2.344	1.00	18.04	A
ATOM	1063	H	GLY	226	-4.218	3.616	1.813	1.00	15.00	A
ATOM	1064	CA	GLY	226	-4.541	2.833	3.841	1.00	18.37	A
ATOM	1065	C	GLY	226	-4.193	4.171	4.544	1.00	17.08	A
ATOM	1066	O	GLY	226	-3.389	4.906	4.055	1.00	13.75	A
ATOM	1067	N	GLY	227	-4.781	4.457	5.725	1.00	16.30	A
ATOM	1068	H	GLY	227	-5.434	3.771	6.036	1.00	15.00	A
ATOM	1069	CA	GLY	227	-4.379	5.649	6.490	1.00	8.52	A
ATOM	1070	C	GLY	227	-4.935	5.631	7.959	1.00	12.75	A
ATOM	1071	O	GLY	227	-5.651	4.748	8.466	1.00	10.57	A
ATOM	1072	N	VAL	228	-4.588	6.698	8.675	1.00	9.23	A
ATOM	1073	H	VAL	228	-4.040	7.398	8.222	1.00	15.00	A
ATOM	1074	CA	VAL	228	-5.110	6.818	10.067	1.00	11.74	A
ATOM	1075	CB	VAL	228	-4.085	7.320	11.144	1.00	14.30	A
ATOM	1076	CG1	VAL	228	-2.830	6.445	11.333	1.00	10.73	A
ATOM	1077	CG2	VAL	228	-4.789	7.565	12.479	1.00	17.07	A
ATOM	1078	C	VAL	228	-6.238	7.803	10.098	1.00	9.03	A
ATOM	1079	O	VAL	228	-6.089	8.937	9.649	1.00	12.01	A

FIGURE 17S

ATOM	1080	N	PHE	229	-7.347	7.299	10.640	1.00	8.88	A
ATOM	1081	H	PHE	229	-7.329	6.332	10.922	1.00	15.00	A
ATOM	1082	CA	PHE	229	-8.566	9.106	10.772	1.00	11.18	A
ATOM	1083	CB	PHE	229	-9.578	7.687	9.686	1.00	8.11	A
ATOM	1084	CG	PHE	229	-9.063	7.912	8.233	1.00	8.40	A
ATOM	1085	CD1	PHE	229	-9.140	9.196	7.649	1.00	10.03	A
ATOM	1086	CD2	PHE	229	-8.433	6.883	7.517	1.00	6.57	A
ATOM	1087	CE1	PHE	229	-8.512	9.443	6.395	1.00	5.18	A
ATOM	1088	CE2	PHE	229	-7.771	7.128	6.282	1.00	4 26	A
ATOM	1089	CZ	PHE	229	-7.813	8.424	5.731	1.00	5.71	A
ATOM	1090	C	PHE	229	-9.202	8.014	12.197	1.00	14 39	A
ATOM	1091	O	PHE	229	-9.116	7.000	12.870	1.00	13 92	A
ATOM	1092	N	GLU	230	-9.863	9.064	12.672	1.00	17.93	A
ATOM	1093	H	GLU	230	-9.912	9.892	12.113	1.00	15.00	A
ATOM	1094	CA	GLU	230	-10.856	8.944	13.770	1.00	18 08	A
ATOM	1095	CB	GLU	230	-11.218	10.303	14.393	1.00	16 17	A
ATOM	1096	CG	GLU	230	-11.068	10.090	15.889	1.00	27.69	A
ATOM	1097	CD	GLU	230	-12.314	10.091	16.805	1.00	33.06	A
ATOM	1098	OE1	GLU	230	-13.355	10.707	16.552	1.00	38.26	A
ATOM	1099	OE2	GLU	230	-12.218	9.477	17.863	1.00	38.14	A
ATOM	1100	C	GLU	230	-12.225	8.268	13.453	1.00	18.70	A
ATOM	1101	O	GLU	230	-12.967	8.519	12.492	1.00	21.58	A
ATOM	1102	N	LEU	231	-12.542	7.334	14.361	1.00	13.79	A
ATOM	1103	H	LEU	231	-11.840	7.125	15.015	1.00	15.00	A
ATOM	1104	CA	LEU	231	-13.885	6.836	14.330	1.00	13.52	A
ATOM	1105	CB	LEU	231	-13.954	5.378	14.002	1.00	13.90	A
ATOM	1106	CG	LEU	231	-13.199	5.064	12.725	1.00	15.44	A
ATOM	1107	CD1	LEU	231	-13.781	5.712	11.436	1.00	10.24	A
ATOM	1108	CD2	LEU	231	-12.970	3.569	12.769	1.00	11.74	A
ATOM	1109	C	LEU	231	-14.638	7.074	15.591	1.00	14.88	A
ATOM	1110	O	LEU	231	-14.145	6.912	16.692	1.00	12.46	A
ATOM	1111	N	GLN	232	-15.891	7.411	15.350	1.00	19.40	A
ATOM	1112	H	GLN	232	-16.107	7.560	14.394	1.00	15.00	A
ATOM	1113	CA	GLN	232	-16.920	7.509	16.389	1.00	21.07	A
ATOM	1114	CB	GLN	232	-18.132	8.234	15.804	1.00	23.55	A
ATOM	1115	CG	GLN	232	-17.792	9.709	15.687	1.00	28.60	A
ATOM	1116	CD	GLN	232	-17.625	10.200	17.102	1.00	33.66	A
ATOM	1117	OE1	GLN	232	-18.623	10.472	17.742	1.00	38.08	A
ATOM	1118	NE2	GLN	232	-16.380	10.254	17.596	1.00	33.41	A
ATOM	1119	HE21	GLN	232	-15.596	10.186	16.972	1.00	15.00	A
ATOM	1120	HE22	GLN	232	-16.387	10.470	18.576	1.00	15.00	A
ATOM	1121	C	GLN	232	-17.402	6.148	16.851	1.00	21.86	A
ATOM	1122	O	GLN	232	-17 368	5.218	16.052	1.00	21.58	A
ATOM	1123	N	PRO	233	-17.906	6.013	18.115	1.00	22.31	A
ATOM	1124	CD	PRO	233	-17 962	7.033	19.168	1.00	21.41	A
ATOM	1125	CA	PRO	233	-18 570	4.747	18.442	1.00	21.21	A
ATOM	1126	CB	PRO	233	-19.013	4.987	19.866	1.00	23.88	A
ATOM	1127	CG	PRO	233	-18.661	6.404	20.339	1.00	20.95	A
ATOM	1128	C	PRO	233	-19.667	4.417	17.434	1.00	23.66	A
ATOM	1129	O	PRO	233	-20 275	5.219	16.875	1.00	26.89	A
ATOM	1130	N	GLY	234	-19.731	3.140	17.059	1.00	22.77	A
ATOM	1131	H	GLY	234	-19.082	2.466	17.417	1.00	15.00	A
ATOM	1132	CA	GLY	234	-20.766	2.767	16.072	1.00	19.45	A
ATOM	1133	C	GLY	234	-20.545	3.241	14.625	1.00	19.67	A
ATOM	1134	O	GLY	234	-21.299	2.980	13.715	1.00	23.81	A
ATOM	1135	N	ALA	235	-19.405	3.926	14.368	1.00	18 89	A
ATOM	1136	H	ALA	235	-19.096	4.485	15.135	1.00	15.00	A
ATOM	1137	CA	ALA	235	-18.431	3.515	13.296	1.00	22.17	A
ATOM	1138	CB	ALA	235	-18.193	2.042	13.039	1.00	6.68	A
ATOM	1139	C	ALA	235	-18 540	4.160	11.993	1.00	21.96	A

FIGURE 17T

ATOM	1140	O	ALA	235	-18.486	5.385	10.100	1.00	26.40	A
ATOM	1141	N	SER	236	-18.699	3.496	10.787	1.00	22.34	A
ATOM	1142	H	SER	236	-18.624	4.326	10.254	1.00	15.00	A
ATOM	1143	CA	SER	236	-18.630	2.227	9.961	1.00	17.80	A
ATOM	1144	CB	SER	236	-19.905	1.676	9.160	1.00	14.96	A
ATOM	1145	OG	SER	236	-20.662	0.908	9.833	1.00	21.35	A
ATOM	1146	HG	SER	236	-21.599	0.910	9.647	1.00	15.00	A
ATOM	1147	C	SER	236	-17.794	2.538	8.714	1.00	13.65	A
ATOM	1148	O	SER	236	-17.939	3.614	8.131	1.00	16.29	A
ATOM	1149	N	VAL	237	-16.986	1.567	8.286	1.00	14.95	A
ATOM	1150	H	VAL	237	-16.764	0.823	8.949	1.00	15.00	A
ATOM	1151	CA	VAL	237	-16.201	1.802	7.077	1.00	11.42	A
ATOM	1152	CB	VAL	237	-14.681	2.004	7.284	1.00	12.49	A
ATOM	1153	CG1	VAL	237	-14.113	0.726	7.939	1.00	13.10	A
ATOM	1154	CG2	VAL	237	-14.254	3.396	7.846	1.00	10.27	A
ATOM	1155	C	VAL	237	-16.468	0.746	6.035	1.00	8.76	A
ATOM	1156	O	VAL	237	-16.827	-0.363	6.341	1.00	12.84	A
ATOM	1157	N	PHE	238	-16.354	1.158	4.773	1.00	12.45	A
ATOM	1158	H	PHE	238	-16.139	2.128	4.652	1.00	15.00	A
ATOM	1159	CA	PHE	238	-16.521	0.213	3.653	1.00	11.21	A
ATOM	1160	CB	PHE	238	-18.013	0.137	3.322	1.00	13.00	A
ATOM	1161	CG	PHE	238	-18.634	1.468	2.899	1.00	12.17	A
ATOM	1162	CD1	PHE	238	-18.763	1.812	1.518	1.00	12.94	A
ATOM	1163	CD2	PHE	238	-19.135	2.332	3.887	1.00	10.55	A
ATOM	1164	CE1	PHE	238	-19.407	3.010	1.092	1.00	14.01	A
ATOM	1165	CE2	PHE	238	-19.786	3.504	3.470	1.00	12.74	A
ATOM	1166	CZ	PHE	238	-19.917	3.836	2.100	1.00	13.17	A
ATOM	1167	C	PHE	238	-15.725	0.582	2.379	1.00	11.20	A
ATOM	1168	O	PHE	238	-15.137	1.638	2.267	1.00	8.73	A
ATOM	1169	N	VAL	239	-15.726	-0.300	1.383	1.00	14.34	A
ATOM	1170	H	VAL	239	-16.187	-1.170	1.523	1.00	15.00	A
ATOM	1171	CA	VAL	239	-14.982	0.027	0.154	1.00	14.65	A
ATOM	1172	CB	VAL	239	-13.900	-1.043	-0.162	1.00	14.09	A
ATOM	1173	CG1	VAL	239	-13.004	-1.318	1.038	1.00	14.55	A
ATOM	1174	CG2	VAL	239	-13.064	-0.594	-1.361	1.00	14.74	A
ATOM	1175	C	VAL	239	-15.930	0.081	-1.043	1.00	18.32	A
ATOM	1176	O	VAL	239	-16.558	-0.903	-1.369	1.00	18.99	A
ATOM	1177	N	ASN	240	-16.000	1.207	-1.707	1.00	19.26	A
ATOM	1178	H	ASN	240	-15.420	1.947	-1.383	1.00	15.00	A
ATOM	1179	CA	ASN	240	-16.613	1.355	-3.031	1.00	21.66	A
ATOM	1180	CB	ASN	240	-16.850	2.856	-3.095	1.00	24.58	A
ATOM	1181	CG	ASN	240	-18.167	3.077	-3.708	1.00	29.09	A
ATOM	1182	OD1	ASN	240	-18.948	2.123	-3.740	1.00	35.44	A
ATOM	1183	ND2	ASN	240	-18.293	4.331	-4.166	1.00	34.71	A
ATOM	1184	HD21	ASN	240	-19.149	4.489	-4.657	1.00	15.00	A
ATOM	1185	C	ASN	240	-15.669	0.950	-4.184	1.00	20.96	A
ATOM	1186	O	ASN	240	-14.473	1.128	-4.058	1.00	20.99	A
ATOM	1187	N	VAL	241	-16.189	0.383	-5.275	1.00	21.52	A
ATOM	1188	H	VAL	241	-17.182	0.230	-5.295	1.00	15.00	A
ATOM	1189	CA	VAL	241	-15.387	0.439	-6.516	1.00	20.56	A
ATOM	1190	CB	VAL	241	-14.581	-0.850	-6.849	1.00	18.02	A
ATOM	1191	CG1	VAL	241	-15.501	-2.058	-7.063	1.00	15.06	A
ATOM	1192	CG2	VAL	241	-13.597	-1.259	-5.764	1.00	20.05	A
ATOM	1193	C	VAL	241	-16.253	0.758	-7.741	1.00	18.88	A
ATOM	1194	O	VAL	241	-17.441	0.500	-7.819	1.00	18.63	A
ATOM	1195	N	THR	242	-15.541	1.162	-8.762	1.00	21.24	A
ATOM	1196	H	THR	242	-14.704	1.653	-8.486	1.00	15.00	A
ATOM	1197	CA	THR	242	-16.246	1.476	-10.031	1.00	20.63	A
ATOM	1198	CB	THR	242	-15.342	2.269	-10.981	1.00	15.80	A
ATOM	1199	OG1	THR	242	-14.035	1.663	-10.953	1.00	17.72	A

FIGURE 17U

ATOM	1200	HG1	THR	242	-13.721	1.969	-11.812	1.00	15.00	A
ATOM	1201	CG2	THR	242	-15.238	3.732	-10.650	1.00	18.24	A
ATOM	1202	C	THR	242	-16.755	1.240	-10.783	1.00	18.91	A
ATOM	1203	O	THR	242	-17.846	0.198	-11.297	1.00	21.25	A
ATOM	1204	N	ASP	243	-15.923	-0.806	-10.718	1.00	21.98	A
ATOM	1205	H	ASP	243	-15.087	-0.580	-10.221	1.00	15.00	A
ATOM	1206	CA	ASP	243	-16.092	-1.977	-11.628	1.00	21.28	A
ATOM	1207	CB	ASP	243	-14.905	-2.126	-12.594	1.00	22.05	A
ATOM	1208	CG	ASP	243	-14.932	-0.954	-13.492	1.00	28.23	A
ATOM	1209	OD1	ASP	243	-14.314	0.051	-13.115	1.00	28.43	A
ATOM	1210	OD2	ASP	243	-15.588	-1.033	-14.535	1.00	33.00	A
ATOM	1211	C	ASP	243	-16.123	-3.308	-10.923	1.00	20.38	A
ATOM	1212	O	ASP	243	-15.146	-4.072	-10.967	1.00	20.43	A
ATOM	1213	N	PRO	244	-17.204	-3.553	-10.154	1.00	19.92	A
ATOM	1214	CD	PRO	244	-18.481	-2.871	-10.071	1.00	16.83	A
ATOM	1215	CA	PRO	244	-17.120	-4.706	-9.269	1.00	19.13	A
ATOM	1216	CB	PRO	244	-18.293	-4.535	-8.275	1.00	15.33	A
ATOM	1217	CG	PRO	244	-18.890	-3.174	-8.634	1.00	15.21	A
ATOM	1218	C	PRO	244	-16.975	-6.034	-9.974	1.00	19.29	A
ATOM	1219	O	PRO	244	-16.194	-6.859	-9.548	1.00	23.48	A
ATOM	1220	N	SER	245	-17.581	-6.163	-11.150	1.00	22.60	A
ATOM	1221	H	SER	245	-18.220	-5.459	-11.473	1.00	15.00	A
ATOM	1222	CA	SER	245	-17.414	-7.429	-11.942	1.00	25.50	A
ATOM	1223	CB	SER	245	-18.256	-7.369	-13.234	1.00	21.36	A
ATOM	1224	OG	SER	245	-19.667	-7.567	-12.981	1.00	38.26	A
ATOM	1225	HG	SER	245	-19.848	-7.390	-12.038	1.00	15.00	A
ATOM	1226	C	SER	245	-15.955	-7.776	-12.328	1.00	24.14	A
ATOM	1227	O	SER	245	-15.477	-8.859	-12.623	1.00	24.84	A
ATOM	1228	N	GLN	246	-15.177	-6.689	-12.385	1.00	28.52	A
ATOM	1229	H	GLN	246	-15.638	-5.804	-12.265	1.00	15.00	A
ATOM	1230	CA	GLN	246	-13.743	-6.923	-12.590	1.00	26.45	A
ATOM	1231	CB	GLN	246	-13.144	-5.645	-13.233	1.00	29.90	A
ATOM	1232	CG	GLN	246	-13.403	-5.435	-14.758	1.00	26.84	A
ATOM	1233	CD	GLN	246	-14.862	-5.341	-15.129	1.00	21.60	A
ATOM	1234	OE1	GLN	246	-15.538	-4.503	-14.616	1.00	24.20	A
ATOM	1235	NE2	GLN	246	-15.334	-6.234	-15.975	1.00	26.15	A
ATOM	1236	HE21	GLN	246	-14.763	-6.924	-16.423	1.00	15.00	A
ATOM	1237	HE22	GLN	246	-16.320	-6.119	-16.084	1.00	15.00	A
ATOM	1238	C	GLN	246	-12.936	-7.372	-11.363	1.00	27.14	A
ATOM	1239	O	GLN	246	-11.721	-7.570	-11.454	1.00	25.73	A
ATOM	1240	N	VAL	247	-13.615	-7.395	-10.196	1.00	23.70	A
ATOM	1241	H	VAL	247	-14.600	-7.594	-10.146	1.00	15.00	A
ATOM	1242	CA	VAL	247	-12.728	-7.569	-9.097	1.00	21.91	A
ATOM	1243	CB	VAL	247	-13.156	-6.814	-7.859	1.00	21.59	A
ATOM	1244	CG1	VAL	247	-14.027	-7.616	-6.962	1.00	24.52	A
ATOM	1245	CG2	VAL	247	-13.690	-5.409	-8.167	1.00	21.61	A
ATOM	1246	C	VAL	247	-12.258	-8.998	-8.910	1.00	21.55	A
ATOM	1247	O	VAL	247	-12.946	-9.912	9.251	1.00	19.53	A
ATOM	1248	N	SER	248	-11.000	-9.152	-8.444	1.00	21.31	A
ATOM	1249	H	SER	248	-10.558	-8.342	-8.070	1.00	15.00	A
ATOM	1250	CA	SER	248	-10.414	-10.499	-8.327	1.00	21.97	A
ATOM	1251	CB	SER	248	-8.939	-10.571	-8.828	1.00	23.61	A
ATOM	1252	OG	SER	248	-8.860	-9.952	-10.128	1.00	20.21	A
ATOM	1253	HG	SER	248	-9.752	-10.027	-10.496	1.00	15.00	A
ATOM	1254	C	SER	248	-10.538	-11.076	-6.946	1.00	19.28	A
ATOM	1255	O	SER	248	-10.048	-10.409	-6.052	1.00	20.64	A
ATOM	1256	N	HIS	249	-11.269	-12.204	-6.814	1.00	18.72	A
ATOM	1257	H	HIS	249	-11.284	-12.753	-7.674	1.00	15.00	A
ATOM	1258	CA	HIS	249	-11.640	-12.673	-5.478	1.00	17.22	A
ATOM	1259	CB	HIS	249	-13.080	-13.152	-5.484	1.00	13.10	A

FIGURE 17V

ATOM	1260	CG	HIS	249	-13.919	-11.905	-5.550	1.00	[illegible]	A
ATOM	1261	ND1	HIS	249	-14.137	-11.129	-4.486	1.00	13.47	A
ATOM	1262	HD1	HIS	249	-13.720	-11.294	-3.611	1.00	15.00	A
ATOM	1263	CD2	HIS	249	-14.662	-11.414	-6.610	1.00	11.61	A
ATOM	1264	NE2	HIS	249	-15.317	-10.347	-6.134	1.00	18.51	A
ATOM	1265	CE1	HIS	249	-15.018	-10.142	-4.821	1.00	12.36	A
ATOM	1266	C	HIS	249	-10.701	-13.683	-4.858	1.00	23.58	A
ATOM	1267	O	HIS	249	-11.103	-14.729	-4.359	1.00	21.98	A
ATOM	1268	N	GLY	250	-9.398	-12.258	-4.878	1.00	29.10	A
ATOM	1269	H	GLY	250	-9.252	-12.351	-5.253	1.00	15.00	A
ATOM	1270	CA	GLY	250	-8.410	-14.041	-4.115	1.00	24.27	A
ATOM	1271	C	GLY	250	-8.336	-15.372	-4.743	1.00	25.93	A
ATOM	1272	O	GLY	250	-8.940	-15.520	-5.795	1.00	29.26	A
ATOM	1273	N	THR	251	-7.594	-16.302	-4.127	1.00	22.38	A
ATOM	1274	H	THR	251	-7.485	-17.038	-4.804	1.00	15.00	A
ATOM	1275	CA	THR	251	-7.111	-16.139	-2.725	1.00	21.12	A
ATOM	1276	CB	THR	251	-6.988	-17.525	-1.933	1.00	24.76	A
ATOM	1277	OG1	THR	251	-5.877	-17.641	-0.981	1.00	22.90	A
ATOM	1278	HG1	THR	251	-6.063	-18.366	-0.381	1.00	15.00	A
ATOM	1279	CG2	THR	251	-6.968	-18.722	-2.890	1.00	22.77	A
ATOM	1280	C	THR	251	-5.952	-15.158	-2.473	1.00	17.96	A
ATOM	1281	O	THR	251	-4.969	-15.043	-3.213	1.00	12.30	A
ATOM	1282	N	GLY	252	-6.241	-14.367	-1.419	1.00	16.85	A
ATOM	1283	H	GLY	252	-7.093	-14.432	-0.862	1.00	15.00	A
ATOM	1284	CA	GLY	252	-5.277	-13.375	-0.928	1.00	13.16	A
ATOM	1285	C	GLY	252	-5.357	-12.058	-1.670	1.00	15.51	A
ATOM	1286	O	GLY	252	-4.580	-11.168	-1.439	1.00	15.18	A
ATOM	1287	N	PHE	253	-6.189	-12.063	-2.744	1.00	16.66	A
ATOM	1288	H	PHE	253	-6.868	-12.805	-2.761	1.00	15.00	A
ATOM	1289	CA	PHE	253	-6.110	-10.892	-3.651	1.00	15.77	A
ATOM	1290	CB	PHE	253	-6.649	-11.216	-5.100	1.00	17.11	A
ATOM	1291	CG	PHE	253	-5.595	-11.840	-5.994	1.00	11.82	A
ATOM	1292	CD1	PHE	253	-4.385	-11.175	-6.231	1.00	13.69	A
ATOM	1293	CD2	PHE	253	-5.845	-13.089	-6.558	1.00	18.59	A
ATOM	1294	CE1	PHE	253	-3.364	-11.771	-6.993	1.00	14.39	A
ATOM	1295	CE2	PHE	253	-4.840	-13.680	-7.363	1.00	21.37	A
ATOM	1296	CZ	PHE	253	-3.612	-13.014	-7.562	1.00	15.72	A
ATOM	1297	C	PHE	253	-6.740	-9.599	-3.147	1.00	13.88	A
ATOM	1298	O	PHE	253	-6.347	-8.477	-3.453	1.00	14.27	A
ATOM	1299	N	THR	254	-7.865	-9.837	-2.502	1.00	14.00	A
ATOM	1300	H	THR	254	-8.079	-10.748	-2.124	1.00	15.00	A
ATOM	1301	CA	THR	254	-8.741	-8.681	-2.185	1.00	14.09	A
ATOM	1302	CB	THR	254	-9.908	-8.469	-3.201	1.00	11.66	A
ATOM	1303	OG1	THR	254	-9.414	-8.325	-4.536	1.00	13.08	A
ATOM	1304	HG1	THR	254	-9.826	-9.054	-4.992	1.00	15.00	A
ATOM	1305	CG2	THR	254	-10.882	-7.321	-2.885	1.00	13.78	A
ATOM	1306	C	THR	254	-9.270	-8.779	-0.738	1.00	12.36	A
ATOM	1307	O	THR	254	-9.906	-9.695	-0.240	1.00	14.54	A
ATOM	1308	N	SER	255	-9.007	-7.683	-0.027	1.00	13.42	A
ATOM	1309	H	SER	255	-8.425	-7.021	-0.490	1.00	15.00	A
ATOM	1310	CA	SER	255	-9.032	-7.725	1.431	1.00	7.59	A
ATOM	1311	CB	SER	255	-7.793	-8.466	1.976	1.00	6.39	A
ATOM	1312	OG	SER	255	-6.704	-7.560	2.041	1.00	9.69	A
ATOM	1313	HG	SER	255	-5.920	-8.031	1.741	1.00	15.00	A
ATOM	1314	C	SER	255	-9.248	-6.341	2.085	1.00	10.05	A
ATOM	1315	O	SER	255	-9.191	-5.254	1.492	1.00	15.21	A
ATOM	1316	N	PHE	256	-9.653	-6.365	3.369	1.00	8.54	A
ATOM	1317	H	PHE	256	-9.710	-7.323	3.733	1.00	15.00	A
ATOM	1318	CA	PHE	256	-10.114	-6.168	4.035	1.00	7.94	A
ATOM	1319	CB	PHE	256	-11.605	-6.009	3.679	1.00	11.65	A

FIGURE 17W

ATOM	1320	CG	PHE	256	-12.376	-3.524	4.235	1.00	8.72	A
ATOM	1321	CD1	PHE	256	-11.766	-2.570	4.533	1.00	11.20	A
ATOM	1322	CD2	PHE	256	-13.756	-3.976	4.327	1.00	6.11	A
ATOM	1323	CE1	PHE	256	-12.503	-1.490	5.034	1.00	11.49	A
ATOM	1324	CE2	PHE	256	-14.514	-2.849	4.734	1.00	8.58	A
ATOM	1325	CZ	PHE	256	-13.862	-1.657	5.211	1.00	9.27	A
ATOM	1326	C	PHE	256	-9.933	-5.268	5.560	1.00	11.92	A
ATOM	1327	O	PHE	256	-10.195	-6.290	6.177	1.00	9.43	A
ATOM	1328	N	GLY	257	-9.420	-4.207	6.169	1.00	10.57	A
ATOM	1329	H	GLY	257	-9.217	-3.365	5.653	1.00	15.00	A
ATOM	1330	CA	GLY	257	-9.368	-4.406	7.612	1.00	11.26	A
ATOM	1331	C	GLY	257	-8.965	-3.122	8.287	1.00	11.14	A
ATOM	1332	O	GLY	257	-8.916	-2.068	7.679	1.00	10.81	A
ATOM	1333	N	LEU	258	-8.688	-3.277	9.565	1.00	12.61	A
ATOM	1334	H	LEU	258	-8.776	-4.204	9.943	1.00	15.00	A
ATOM	1335	CA	LEU	258	-8.434	-2.098	10.426	1.00	14.72	A
ATOM	1336	CB	LEU	258	-9.751	-1.212	10.704	1.00	14.67	A
ATOM	1337	CG	LEU	258	-10.991	-1.863	11.379	1.00	18.02	A
ATOM	1338	CD1	LEU	258	-12.317	-1.125	11.094	1.00	15.05	A
ATOM	1339	CD2	LEU	258	-10.743	-2.047	12.905	1.00	15.42	A
ATOM	1340	C	LEU	258	-7.737	-2.525	11.709	1.00	11.84	A
ATOM	1341	O	LEU	258	-7.851	-3.690	12.096	1.00	7.91	A
ATOM	1342	N	LEU	259	-7.058	-1.537	12.343	1.00	11.64	A
ATOM	1343	H	LEU	259	-6.883	-0.685	11.844	1.00	15.00	A
ATOM	1344	CA	LEU	259	-6.581	-1.780	13.714	1.00	9.53	A
ATOM	1345	CB	LEU	259	-5.155	-2.417	13.831	1.00	7.40	A
ATOM	1346	CG	LEU	259	-4.194	-1.621	12.931	1.00	11.40	A
ATOM	1347	CD1	LEU	259	-3.355	-2.412	11.926	1.00	7.83	A
ATOM	1348	CD2	LEU	259	-3.379	-0.670	13.808	1.00	13.30	A
ATOM	1349	C	LEU	259	-6.652	-0.497	14.531	1.00	10.40	A
ATOM	1350	O	LEU	259	-6.202	0.556	14.082	1.00	9.73	A
ATOM	1351	N	LYS	260	-7.193	-0.629	15.762	1.00	12.00	A
ATOM	1352	H	LYS	260	-7.395	-1.553	16.115	1.00	15.00	A
ATOM	1353	CA	LYS	260	-7.069	0.521	16.693	1.00	13.51	A
ATOM	1354	CB	LYS	260	-8.024	0.312	17.885	1.00	13.49	A
ATOM	1355	CG	LYS	260	-8.378	1.656	18.521	1.00	17.16	A
ATOM	1356	CD	LYS	260	-9.435	1.456	19.596	1.00	12.01	A
ATOM	1357	CE	LYS	260	-10.151	2.681	20.121	1.00	11.41	A
ATOM	1358	NZ	LYS	260	-9.175	3.595	20.697	1.00	13.33	A
ATOM	1359	HZ1	LYS	260	-8.534	3.932	19.954	1.00	15.00	A
ATOM	1360	HZ2	LYS	260	-9.693	4.404	21.095	1.00	15.00	A
ATOM	1361	HZ3	LYS	260	-8.638	3.136	21.458	1.00	15.00	A
ATOM	1362	C	LYS	260	-5.649	0.921	17.125	1.00	16.54	A
ATOM	1363	O	LYS	260	-4.828	0.112	17.481	1.00	15.61	A
ATOM	1364	N	LEU	261	-5.353	2.199	17.015	1.00	14.78	A
ATOM	1365	H	LEU	261	-6.089	2.638	16.856	1.00	15.00	A
ATOM	1366	CB	LEU	261	-3.705	4.005	17.185	1.00	19.53	A
ATOM	1367	CG	LEU	261	-3.177	4.309	15.787	1.00	16.82	A
ATOM	1368	CD1	LEU	261	-3.010	5.779	15.767	1.00	12.45	A
ATOM	1369	CD2	LEU	261	-4.010	3.906	14.577	1.00	18.20	A
ATOM	1370	C	LEU	261	-4.243	2.667	19.225	1.00	20.80	A
ATOM	1371	OCT1	LEU	261	-5.363	2.741	19.746	1.00	22.59	A
ATOM	1372	OCT2	LEU	261	-3.221	2.696	19.913	1.00	26.97	A
ATOM	1373	CA	LEU	261	-4.122	2.604	17.684	1.00	18.13	A
ATOM	1374	O	HOH	501	-20.040	9.837	7.596	1.00	16.33	W
ATOM	1375	H1	HOH	501	-19.411	10.547	7.803	1.00	10.00	W
ATOM	1376	H2	HOH	501	-19.615	9.317	6.900	1.00	10.00	W
ATOM	1377	O	HOH	502	-9.727	11.545	10.743	1.00	10.94	W
ATOM	1378	H1	HOH	502	-10.039	11.934	9.919	1.00	15.00	W
ATOM	1379	H2	HOH	502	-10.233	12.125	11.325	1.00	15.00	W

FIGURE 17X

ATOM	1380	O	HOH	503	-8.158	13.188	13.681	1.00	30.64	W
ATOM	1381	H1	HOH	503	-8.715	12.529	13.277	1.00	15.00	W
ATOM	1382	H2	HOH	503	-8.700	13.944	13.574	1.00	15.00	W
ATOM	1383	O	HOH	504	-16.772	8.440	12.789	1.00	10.00	W
ATOM	1384	H1	HOH	504	-17.194	9.259	12.886	1.00	10.00	W
ATOM	1385	H2	HOH	504	-15.921	8.763	12.582	1.00	10.00	W
ATOM	1386	O	HOH	505	-25.173	7.297	7.925	1.00	47.03	W
ATOM	1387	H1	HOH	505	-24.690	8.064	8.239	1.00	10.00	W
ATOM	1388	H2	HOH	505	-25.990	7.684	7.583	1.00	10.00	W
ATOM	1389	O	HOH	506	-23.612	14.948	13.859	1.00	36.14	W
ATOM	1390	H1	HOH	506	-24.160	15.702	13.605	1.00	10.00	W
ATOM	1391	H2	HOH	506	-23.282	15.191	14.748	1.00	10.00	W
ATOM	1392	O	HOH	507	-17.329	-8.460	-7.186	1.00	34.02	W
ATOM	1393	O	HOH	508	-18.687	-7.253	-3.843	1.00	63.14	W
ATOM	1394	O	HOH	509	-7.157	11.327	3.239	1.00	22.26	W
ATOM	1395	O	HOH	510	-19.322	7.486	-2.227	1.00	37.69	W
ATOM	1396	O	HOH	511	-14.645	-7.711	-1.931	1.00	26.48	W
ATOM	1397	O	HOH	512	-18.377	-9.754	12.556	1.00	24.86	W
ATOM	1398	O	HOH	513	0.030	0.048	-13.455	1.00	26.05	W
ATOM	1399	O	HOH	514	-8.938	5.945	22.862	1.00	34.39	W
ATOM	1400	O	HOH	515	-29.446	-4.922	-7.247	1.00	41.61	W
ATOM	1401	O	HOH	516	-12.982	10.220	10.038	1.00	47.16	W
ATOM	1402	O	HOH	517	-21.797	-9.377	7.242	1.00	60.65	W
ATOM	1403	O	HOH	518	-7.867	8.165	19.484	1.00	40.46	W
ATOM	1404	O	HOH	520	-15.588	-14.701	14.628	1.00	63.80	W
ATOM	1405	O	HOH	521	-21.844	7.778	20.415	1.00	35.72	W
ATOM	1406	O	HOH	522	-6.555	-3.308	-15.790	1.00	33.63	W
ATOM	1407	O	HOH	523	-9.046	-13.476	-8.051	1.00	44.08	W
ATOM	1408	O	HOH	524	-17.413	-9.311	17.071	1.00	34.06	W
ATOM	1409	O	HOH	525	-23.838	4.781	19.884	1.00	37.99	W
ATOM	1410	O	HOH	526	-26.323	15.525	10.379	1.00	72.49	W
ATOM	1411	O	HOH	527	-3.167	-13.749	-10.820	1.00	43.99	W
ATOM	1412	O	HOH	528	-0.470	2.513	17.943	1.00	63.68	W
ATOM	1413	O	HOH	529	-5.580	-12.778	-14.864	1.00	47.52	W
ATOM	1414	O	HOH	530	-2.641	7.004	2.495	1.00	18.07	W
ATOM	1415	O	HOH	531	-6.472	12.847	0.156	1.00	24.96	W
ATOM	1416	O	HOH	532	-10.363	-16.426	-0.360	1.00	63.56	W
ATOM	1417	O	HOH	533	-1.378	-17.183	-13.053	1.00	67.67	W
ATOM	1418	O	HOH	534	-4.774	9.073	-0.651	1.00	23.36	W
ATOM	1419	O	HOH	535	-18.917	-13.857	6.913	1.00	32.28	W
ATOM	1420	O	HOH	536	-23.062	3.270	0.454	1.00	52.03	W
ATOM	1421	O	HOH	537	-25.906	9.022	16.986	1.00	44.75	W
ATOM	1422	O	HOH	538	-21.729	16.972	17.027	1.00	53.12	W
ATOM	1423	O	HOH	539	-9.084	11.806	17.034	1.00	70.90	W
ATOM	1424	O	HOH	540	-10.938	-13.296	15.207	1.00	35.65	W
ATOM	1425	O	HOH	541	-6.068	13.255	17.989	1.00	67.36	W
ATOM	1426	O	HOH	542	-20.593	-11.039	-9.003	1.00	96.30	W
ATOM	1427	O	HOH	543	-15.926	13.397	1.269	1.00	35.72	W
ATOM	1428	O	HOH	544	-24.591	-7.285	-2.353	1.00	43.42	W
ATOM	1429	O	HOH	545	-25.859	-2.666	-15.747	1.00	53.56	W
ATOM	1430	O	HOH	546	-23.074	-1.533	11.026	1.00	56.44	W
ATOM	1431	O	HOH	548	-8.941	-12.649	-12.394	1.00	64.34	W
ATOM	1432	O	HOH	549	-14.150	6.038	-12.250	1.00	41.38	W
ATOM	1433	O	HOH	550	-14.274	-0.613	18.441	1.00	56.17	W
ATOM	1434	O	HOH	551	-12.241	-19.609	8.637	1.00	80.90	W
ATOM	1435	O	HOH	552	-10.316	15.578	10.166	1.00	39.58	W
ATOM	1436	O	HOH	553	-15.367	10.941	14.659	1.00	40.40	W
ATOM	1437	O	HOH	554	-2.322	1.830	-5.294	1.00	33.65	W
ATOM	1438	O	HOH	555	-22.393	-14.875	-4.217	1.00	52.40	W
ATOM	1439	O	HOH	556	-22.120	14.279	7.189	1.00	38.55	W

FIGURE 17Y

ATOM	1440	O	HOH	557	-28.833	6.135	9.560	1.00	37.40	W
ATOM	1441	O	HOH	558	-5.554	-16.509	13.192	1.00	88.55	W
ATOM	1442	O	HOH	559	-22.996	12.522	1.162	1.00	63.77	W
ATOM	1443	O	HOH	560	-13.764	2.268	-14.743	1.00	27.47	W
ATOM	1444	O	HOH	561	-15.556	7.750	-5.628	1.00	75.88	W
ATOM	1445	O	HOH	562	-1.970	-15.363	-17.719	1.00	76.30	W
ATOM	1446	O	HOH	563	-18.939	-0.335	-13.842	1.00	48.39	W
ATOM	1447	O	HOH	564	-12.619	14.760	-6.974	1.00	100.59	W
ATOM	1448	O	HOH	565	-9.491	16.046	13.682	1.00	87.45	W
ATOM	1449	O	HOH	566	-11.655	-11.140	22.481	1.00	28.88	W
ATOM	1450	O	HOH	567	-24.072	-3.264	-0.332	1.00	35.13	W
ATOM	1451	O	HOH	568	-27.455	0.119	-7.117	1.00	71.07	W
ATOM	1452	O	HOH	569	-14.604	3.516	-6.119	1.00	59.45	W
ATOM	1453	O	HOH	570	-2.635	-9.566	-16.973	1.00	59.09	W
ATOM	1454	O	HOH	571	-18.841	4.066	-7.543	1.00	34.10	W
ATOM	1455	O	HOH	572	-24.996	1.301	17.953	1.00	70.45	W
ATOM	1456	O	HOH	573	-14.666	16.471	8.995	1.00	62.77	W
ATOM	1457	O	HOH	574	-14.786	1.426	10.949	1.00	82.68	W
ATOM	1458	O	HOH	575	-16.584	-14.717	-4.352	1.00	29.09	W
ATOM	1459	O	HOH	576	-16.273	-4.590	6.109	1.00	104.64	W
ATOM	1460	O	HOH	577	-25.471	-0.127	-2.510	1.00	62.74	W
ATOM	1461	O	HOH	578	-7.334	-17.173	19.514	1.00	89.62	W
ATOM	1462	O	HOH	579	-21.060	14.259	19.996	1.00	69.59	W
ATOM	1463	O	HOH	580	-19.286	4.057	-12.816	1.00	60.37	W
ATOM	1464	O	HOH	581	-22.445	-15.840	0.317	1.00	58.24	W
ATOM	1465	O	HOH	582	-22.434	-10.539	12.489	1.00	70.25	W
ATOM	1466	O	HOH	583	-21.327	3.668	-2.500	1.00	39.32	W
ATOM	1467	O	HOH	584	-25.325	5.247	16.919	1.00	41.31	W
ATOM	1468	O	HOH	585	-24.945	-10.718	-2.375	1.00	38.85	W
ATOM	1469	O	HOH	586	-24.342	-13.003	1.927	1.00	70.58	W
ATOM	1470	O	HOH	587	-18.020	11.871	11.358	1.00	64.47	W
ATOM	1471	O	HOH	588	-27.135	6.965	13.151	1.00	53.96	W
ATOM	1472	O	HOH	589	-14.982	-16.230	-2.494	1.00	30.24	W
ATOM	1473	O	HOH	590	-5.646	14.418	-2.232	1.00	41.78	W
ATOM	1474	O	HOH	591	-2.745	-0.153	-17.104	1.00	55.19	W
ATOM	1475	O	HOH	592	-3.397	-7.012	22.477	1.00	59.46	W
ATOM	1476	O	HOH	593	-32.916	-4.705	-4.143	1.00	51.88	W
ATOM	1477	O	HOH	594	-10.913	-18.855	-3.503	1.00	42.29	W
ATOM	1478	O	HOH	595	-24.157	1.821	-6.165	1.00	47.43	W
END										

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19172

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12Q 1/00; G01N 33/53, 33/567; A61K 39/395, 31/00; A01N 37/18
US CL :435/4, 7.1, 7.21; 424/130.1; 514/2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 7.1, 7.21; 424/130.1; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	US 5,474,771 (LEDERMAN et al) 12 December 1995, see entire document.	1-101
Y	WO 94/04570 (HEATH et al) 03 March 1994, see entire document.	1-101
Y	KUNTZ, I.D. Structure-Based Strategies for Drug Design and Discovery. Science. 21 August 1992, Vol. 257, pages 1028-1031	1-101
Y	KARPUSAS, M. 2 angstrom crystal strucutre of an extracellular fragment of human CD40 ligand. Structure. 15 October 1995, Vol. 3, No. 10, pages 1031-1039, see entire document.	1-101

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of mailing of the international search report
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	DURIE, F.H. Prevention of Collagen-Induced Arthritis with an Antibody to gp39, the Ligand for CD40. Science. 03 September 1993, Vol. 261, pages 1328-1330, see entire document.	1-101